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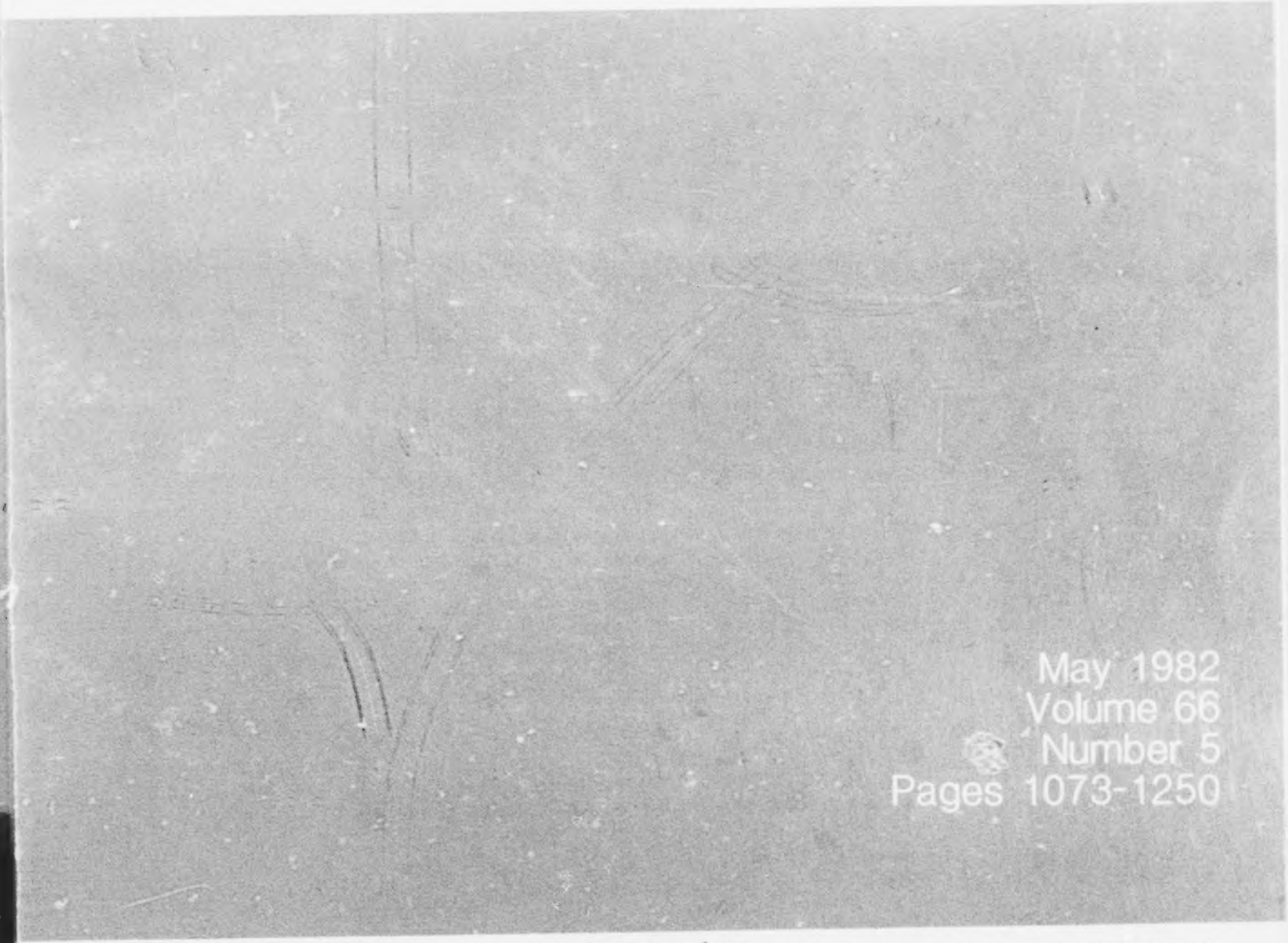

May 1982
Volume 66
Number 5
Pages 1073-1250

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

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COMMENTARY

Chronic Myelocytic Leukemia: Comments on New Approaches to Therapy[1,2]

Harvey D. Preisler* and Azra Raza[3]

The uniformly fatal outcome of chronic myelocytic leukemia (CML) has led to attempts to aggressively treat both the chronic and the blastic phases of the disease. The new approaches include the administration of intensive chemotherapy to ablate the chronic phase Ph^1-containing clone, the use of similar aggressive regimens to suppress what appear to be emerging blastic clones, and the use of allogeneic or isogeneic bone marrow for transplantation during the chronic phase to cure the disease. In addition, attempts have been made to restore the chronic phase of CML through the use of ablative therapy followed by the infusion of stored autologous chronic-phase peripheral blood or marrow. To date, save for the isogeneic marrow transplants, these approaches have met with little success. We will discuss possible reasons for these disappointing results.

SUPPRESSION OF UNWANTED CLONES

Attempts to ablate the Ph^1-containing clone during the chronic phase of CML by administering intensive chemotherapy similar to that used to treat acute myelocytic leukemia (AML) have not proved fruitful (1). Substantial reductions in the proportion of Ph^1-containing cells were obtained in only a minority of patients and, in these, for only a short time. There are at least two reasons for the failure to produce more favorable results. (*a*) For most patients, it is not known whether there are substantial numbers of residual nonleukemic stem cells capable of repopulating the marrow if the Ph^1-containing cell line were significantly suppressed or ablated. (*b*) Even if substantial numbers of normal stem cells are present, there is essentially no information regarding the relative drug sensitivity of the normal and the leukemic clones. Hence, aggressive chemotherapy might, in fact, have greater effects on the residual normal clones and result in their reduction rather than in a reduction of Ph^1-containing cells. We have recently found that the in vitro granulocytic/macrophage clonogenic cells of some patients in the chronic phase of CML are resistant in vitro to a combination of cytarabine and doxorubicin and to as much as 30 μg/ml of cytarabine.[4] Given the good correlation between in vitro drug sensitivity as measured in this assay system and the outcome of remission induction therapy for AML (2), these data suggest that treatment with cytarabine and/or an anthracycline antibiotic would probably have little effect on the Ph^1-containing cells of these patients.

The use of ablative therapy followed by the transplantation of bone marrow obtained from an identical twin or an HLA-matched allogeneic donor has met with some success, in that the Ph^1-containing cell line appears to have been suppressed for long periods of time (3). The use of allogeneic marrow is still a life-threatening procedure, however, because of the toxicity of the preparative regimen and graft-versus-host (GvH) disease. While these studies undoubtedly should be pursued, the attempts at allogeneic transplantation should probably be limited for a time to patients who are at high risk of entering blastic crisis, since the appropriate methodology for this approach is yet to be worked out. A practical problem with respect to determining whether the Ph^1-containing clone is truly absent after transplantation is discussed below.

The appearance of clones which have chromosomal abnormalities in addition to the Ph^1 chromosome has been associated in some cases with the appearance of blastic crisis within a short time (4,5). It has been proposed (and one pilot study has already been carried out) that aggressive chemotherapy should be administered to suppress or ablate

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[1] Received Aug 3, 1981; revised Oct 13, 1981; accepted Dec 7, 1981.

[2] Supported by Public Health Service grants CA-5834 and CA-29380 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Medical Oncology, Roswell Park Memorial Institute, Buffalo, NY.

**Reprint requests to*: Harvey D. Preisler, MD, Department of Medical Oncology, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

[4] Preisler HD. Unpublished observations.

the emerging clones (6). This approach presents two problems. (*a*) The drug sensitivities of the new clones and of the chronic phase clones are unknown; hence, the blind administration of intensive chemotherapy might result in suppression of the very clones that one wanted to preserve. (*b*) Serial cytogenetic studies as usually carried out do not provide a truly early indication of blastic transformation. If the presence of a single cell with a new karyotypic pattern is accepted as being indicative of the appearance of a new clone and if 25 metaphases are characterized, the cells of the new clone can constitute as many as 11% of the metaphases present and still fail to be detected with a 95% probability (7). Fifty metaphases would have to be characterized for a new clone to be detected when it constituted 6% of the metaphases present. In reality, however, if the usual cytogenetic criteria for confirming the presence of an abnormal clone were to be applied, many more metaphases would have to be characterized; the standard cytogenetic criteria stipulate that at least two identical hyperdiploid or three identical hypodiploid cells must be identified before a new clone is said to be present. The exact number of additional metaphases that must be characterized to satisfy these criteria is as yet unknown. Clearly, the same principles and problems apply if one wants to determine whether aggressive therapy has altered the clonal proportions in a cytogenetically mosaic patient or to detect residual or recurrent Ph^1-containing clones in patients who have received bone marrow transplants. In any event, evaluating the usual numbers of metaphases does not permit the "early" detection of blastic crisis. Given the variability of the time to blastic crisis and given the numbers of metaphases that would have to be characterized to discern the effects of aggressive chemotherapy on the proportional representation of the various clones comprising a mosaic, a randomized prospective trial of chemotherapy employing a control group that does not receive intensive chemotherapy is probably the only practical way to determine the clinical merits of such an approach. The cytogenetic evaluation of the efficacy of intensive chemotherapy designed to suppress newly emerging clones is not possible unless a far greater number of metaphases are characterized than is currently practical.

HEMATOPOIETIC TRANSPLANTATION

The principle of reestablishing the chronic phase of CML through the use of autologous hematopoietic transplantation has been clearly established (8). The primary limitation of this approach has been the recurrence of the blastic phase within 3–4 months after transplantation. While the occurrence of a new transforming event has not been ruled out by the use of rigorous cytogenetic studies and cell-surface marker studies, it is generally agreed that inadequate ablation of the blastic clone by the transplantation preparative regimen is responsible for the early recurrence of blastic crisis. Emphasis, therefore, was placed on the use of increasingly aggressive preparative regimens with a consequent reduction in the number of patients who survived the transplantation procedure (9). An alternate approach should be considered. Given the fact that a second chronic phase can be provided with comparative ease by use of a preparative regimen of moderate toxicity (9), emphasis should be placed on posttransplantation therapy during the second chronic phase. The administration of consolidation chemotherapy to suppress the blastic phase clones might result in an increase in the duration of the second chronic phase.

In vitro studies of the drug sensitivity of chronic phase and blastic phase colony-forming cells has revealed a high degree of variability among patients, perhaps reflecting a high mutation rate in the myeloid elements of these patients. If consolidation therapy for transplanted patients in the second chronic phase of their disease is to be administered, attempts should be made to individualize the therapy by taking into account the drug sensitivities of both the blastic phase and the chronic phase clones of each patient. At the time of blastic crisis, the drug sensitivity of the leukemic cells to a variety of agents could be measured. Once the chronic phase of the disease is reestablished by autologous reconstitution, the drug sensitivity of the cells of this phase could also be measured. Alternatively, the drug sensitivity of the chronic phase cells could be determined prior to their infusion into the patient. Intensive consolidation therapy could then be administered utilizing the agents with the highest therapeutic index so that the unwanted clones (residual blastic phase cells) could be maximally suppressed while the chronic phase clones are left relatively unaffected. Intensive leukapheresis at the time of initial diagnosis or even once the second chronic phase is established, with storage of large numbers of chronic phase cells, would permit rescue of patients who had severe hematologic toxic effects from the intensive consolidation therapy. This approach for the selective suppression of persistent aggressive leukemic clones in transplanted patients is clearly applicable to any autologous marrow transplantation for leukemia or for patients with leukemia who receive transplants of allogeneic marrow and are at high risk for leukemic recurrence (10), since posttransplant consolidation chemotherapy regimens in these situations could also be designed on the basis of the relative drug sensitivities of the leukemic clones and of the engrafted healthy marrow.

Parenthetically, an alternate explanation for the apparent beneficial effect of GvH disease in preventing leukemic recurrence is that it may not be due to the GvH disease per se but rather to the "consolidation chemotherapy" that these patients receive when attempts are made to suppress the GvH disease. This possibility has been mentioned in a recent publication (11).

BLASTIC CRISIS

It is widely recognized that the response of blastic phase disease to remission induction chemotherapy is inferior to that of AML. Unfortunately, the nature of the treatment failure has rarely been defined (12,13). There is little or no information regarding the proportion of patients who have persistent leukemia after a particular regimen has been administered as opposed to the proportion who die with hypoplastic bone marrow. Without this information, one cannot determine if a treatment regimen was too aggressive or not aggressive enough and therefore cannot properly evaluate the treatment given.

The recognition of lymphoid crisis has permitted identification of a subset of patients who appear to be more responsive to remission induction therapy. Perhaps a closer examination of the cytogenetic characteristics of patients in myeloid crisis will permit identification of subsets of patients with different degrees of responsiveness to chemotherapy. This statement is based on the following observations. The cytogenetic characteristics of the marrow cells of patients with AML can be used to recognize AML patients whose remission rate is high (NN patients; those in whom only normal metaphases are detected), intermediate (NA patients; those in whom both normal and abnormal metaphases are detected), or low (AA patients; those in whom only abnormal metaphases are detected) (14).[5] Patients in blastic crisis are also heterogeneous with respect to the chromosomal composition of their hematopoietic system; only the chronic phase cytogenetic karyotype is recognized in some, both the chronic phase and new clones are present in others, and only new clones are detectable in still others. These three groups may be comparable, respectively, to the NN, NA, and AA subsets in AML and similarly may manifest different degrees of responsiveness to chemotherapy. Indeed, in myeloid blastic crisis most patients are either NA or AA, and the remission rate for myeloid crisis patients as a group when treated with aggressive therapy is not very different from that for AML patients who are classified as NA or AA. Hence, by classifying blastic crisis patients using approaches which have already been applied to the study of AML, one might be able to identify the subset of patients who do well with chemotherapy and identify the causes of induction failure for the other subset of patients.

As noted above, the recognition of "lymphoid" crisis has permitted identification of a subset of patients whose aggressive phase is more responsive to therapy than most blastic crisis patients. While the cell lines involved clearly appear lymphoid in nature and in many cases have lymphoid markers on the cell surface, it should be remembered that all of the progenitor cells of the myeloid series are lymphoid in appearance, and the proportion of "lymphoid" crises that are not lymphocytic but in fact represent progenitor cell myeloid crises is not yet known. Similarly, the recent report that acute myelofibrosis may represent acute megakaryocytic leukemia with fibrosis secondary to the production of fibrosis-promoting factors by the neoplastic cells (15) suggests that at least some CML patients in whom marrow fibrosis develops may, in fact, be experiencing megakaryocytic transformation. This possibility is significant since the reactive marrow fibrosis associated with acute leukemia is potentially reversible with aggressive chemotherapy (16).

Finally, a major problem in the treatment of CML is the identification of patients who are at high risk for entering blastic crisis, since it is difficult to recommend early aggressive life-threatening therapy to a patient who has a 20%–30% chance of still being in the chronic phase of his illness 5 years after the diagnosis is made (17,18). Projections of the risk for entering crisis based on clinical measurements are not sufficiently reliable to recognize individual high-risk patients (19). It has been suggested that random events lead to mutation and the emergence of the new aggressive clones. If this is the case, then the mutation rate of the myeloid elements in CML patients must be greater than normal. If these events were truly random, then all patients would be at the same risk of entering blastic crisis.

Recent studies have demonstrated that patients with chromosomal instability syndromes have an increased risk for developing neoplastic disease (20). It is likely that the chromosomal instability is associated with an increased risk for mutation. This raises the possibility that patients with CML also have unstable genomes and that at the time of initial diagnosis the degree of instability varies from patient to patient so that the risk of occurrence of mutagenic events associated with the appearance of blastic clones differs between patients. This possibility could be addressed by assessing the genetic stability of a patient's myeloid cells by measuring the sensitivity of these cells to radiation- or chemical mutagen-induced DNA damage and by determining the ability of these cells to repair DNA damage (21,22). Serial studies during the chronic phase of CML might also be useful since patients may evolve into "states" of increased likelihood of mutational events prior to the appearance of blastic clones. Studies of treated patients would obviously be complicated by therapy-induced DNA instability, particularly for those patients treated with busulfan. However, these therapy-induced instability states might, in fact, be clinically relevant. In any event, studies of chromosomal stability might permit recognition of patients who are at high risk for entering blastic crisis. The ability to reliably recognize such patients early in the course of the disease or at the time when the patient evolved into a high-risk state would facilitate the evaluation of the newer aggressive therapeutic approaches to CML.

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[5] Preisler HD, Reese PA, Marinello MJ, et al. Influence of bone marrow cytogenetics on the outcome of therapy for acute nonlymphocytic leukemia. Submitted for publication, 1981.

CONCLUSION

It appears, then, that several recent new approaches to the therapy for CML offer considerable promise. Effective utilization of these new therapeutic possibilities probably depends on the development of newer scientific approaches designed to identify patients for whom such high-risk therapy is appropriate. The chemotherapeutic sensitivity of normal cells, chronic phase CML cells, and blastic phase CML cells must be defined, and new methods must be established for simplifying cytogenetic studies so that large numbers of metaphases can be examined. Finally, the role of posttransplantation consolidation therapy should be studied.

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A Data-Based Approach to Assessing Clinical Interventions in the Setting of Chronic Disease[1,2]

C. Frank Starmer * and Kerry L. Lee [3]

SUMMARY

Accurately assessing the effect of a clinical intervention is a complex task based on observing patients and their outcomes. Two strategies, the randomized trial and the observational data bank, represent different approaches toward detecting an outcome gradient across treatments. The observational data base is rooted on prospective data collected on an unselected group of patients with continuous surveillance of outcome events. As such, this approach captures a broad spectrum of patients within which prognostically important factors can be identified. In addition, the ongoing nature of the observational approach provides a means for capturing the dynamics of the disease process as well as the dynamics of the surrounding technology. These factors are particularly useful in the study of chronic illness where time frames of years to decades are encountered. In assessing treatment effects, both the randomized trial and the observational data base are subject to inferential errors due to multiple comparisons. In this paper, we describe some of the complexity associated with the study of chronic illness and demonstrate the multiple comparison problem. We have found multivariate models helpful in attenuating multiple comparison problems, but it still seems that independent confirmatory studies are required to establish confidence in a questionable intervention.

[Cancer Treat Rep 66:1077–1082, 1982]

BACKGROUND ISSUES

Complex chronic illness dominates the health care scene today, yet its complexity seemingly defies investigation and understanding (1). Chronic illness has several distinctive properties that lead to its complexity and make it difficult to study. Among these properties are a long time course, a sometimes nonstationary disease process, a nonstationary milieu, an unknown etiology, and the frequent requirement for active participation by the patient in the intervention. Further complicating the study of chronic problems is a changing technology that provides new instruments for acquiring more precise descriptors of the illness. It almost seems as if the years-to-decade time frame of a chronic process provides a setting in which Murphy's law goes on a rampage. Anything that can change will change, thereby upsetting the controls necessary for efficient investigation.

It is our belief that the traditional investigative tools, so useful in the study of acute or short-response-time biologic systems, are less suited for the problems surrounding chronic disease. Even operational issues, such as personnel, become areas of concern in studying a system over several years

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[1] Received July 1, 1981; accepted Sept 18, 1981.

[2] Supported in part by Public Health Service grants HS-03834 from the National Center for Health Services Research (Health Resources Administration), HL-17670 from the National Heart, Lung, and Blood Institute (National Institutes of Health), and LM-07003 and LM-03373 from the National Library of Medicine (National Institutes of Health), Department of Health and Human Services; and by grants from the Prudential Insurance Company of America and the Kaiser Family Foundation.

[3] Division of Cardiology, Department of Medicine and Department of Computer Science, Duke University Medical Center, Durham, NC.

****Reprint requests to:*** C. Frank Starmer, PhD, Department of Medicine, Duke University Medical Center, Box 3181, Durham, NC 27710.

because of the cost associated with turnover and motivational problems associated with performing a task on an infrequent basis. For these reasons, we have been integrating traditional statistical methodology with new approaches to data management and analysis in order to deal with problems introduced by temporal complexity. We see little justification in not allowing a study to evolve in time as our understanding evolves and as new measurements become available. We see little justification in assuming a disease process is static over time. We see little justification in ignoring environmental issues.

Our approach to clinical investigation is based on the individual patient, with his care being the ultimate goal. Thus, our activities have been aimed at keeping the practicing physician in touch with an easily accessible repository of experience that is applicable to an individual patient. Summaries of experience derived from subsets of patients characterized in the data base are developed. The interface between the physician and the data base is managed by a group of clinical and biostatistical consultants who "interpret" the requested summaries and review the applicable experiences for such things as subgroup comparability and bias.

We define a data base as a shared repository of data elements describing attributes of patients, interventions, and outcomes. The characterization of each patient includes baseline demographic attributes, properties of organ systems, properties of interventions, and actively acquired periodic followup observations. Data are acquired in line with routine medical care by members of the medical staff. All patients are subjected to periodic followup by either a clinic visit or a telephone call. For our purposes here, it is irrelevant how the data base is actually managed; arbitrarily defined patient data subsets are readily accessible to any user—physician, patient, or investigator. From the perspective of patient care, our goal is to convert a series of clinical experiences with various interventions and patients into a tool for the practicing physician to use with an individual patient, and to provide the patient with a time-lapsed "snapshot" of temporally ordered outcomes of patients similar to himself.

Our focus on individual patient care is the primary motivation for the data base. This point, though seemingly obvious, is not clearly perceived by nonclinicians. We find confusion between the data-based approach to patient care and use of randomized trial data in patient care. The formal randomized clinical trials are focused on a limited number of hypotheses, and their implementation is directed more toward public health issues than patient care issues. In this setting, public health issues, such as polio or flu vaccines, are addressed to aggregates of individuals, with less attention given to those who will react unfavorably. Patient care, in contrast, is individualized, with considerable attention paid to all potential outcomes. Thus, we put considerable weight on identifying homogeneous subgroups of patients from all past experiences, so that an individualized plan for management of a problem can be developed.

CLINICAL SETTING

The setting in which we have chosen to develop our methodology is that of complex chronic illness. As stated earlier, this class of clinical problems is generally characterized by an unknown etiology; a long time course (years to decades) between onset, intervention, and a target outcome; a nonstationary background process (such as the patient's diet and life-style); and an intervention dependent on the patient's active participation (such as long-term medication). The combination of these factors produces a degree of heterogeneity greater than that usually seen in an acute or short-time-frame disease process.

That the patient is an active participant in the intervention raises an additional question when outcome is assessed. Is the intervention effective (favorable outcome with an unstructured intervention where, say, high levels of compliance with a treatment strategy may not be found) or is the intervention efficacious (favorable results from an intervention in the setting of a highly enforced treatment protocol)? We find that the scientific issue (ie, does the intervention work for any highly structured setting?) and the patient care issue (ie, does the intervention work with this patient?) are sometimes addressed as if they were synonymous in the chronic disease setting. Although these two outcome perspectives are frequently equivalent in acute disease, they can be quite different in the chronic setting. The distinction is important, and the differences in expectations from investigators and physicians surely explain much of the controversy surrounding some of the larger clinical trials.

All of these features serve to increase the variability of signs and symptoms in a clinical data base. To accrue the large numbers of patients necessary to yield relatively homogeneous subgroups and to develop robust models from the overall data base, we found it necessary to collect data on all patients. We have been studying coronary artery disease since 1969 and have accumulated data on 6519 patients (5819 having significant coronary artery disease), with followup through 11 years. Data are acquired through checklists filled out by members of the clinical staff as the primary document for the medical record. Data items from these forms (medical history, physical examination, catheterization report, operative note, etc) are entered into the data base, and reports are generated for inclusion in the chart. Quality control is based in part on shared use of common data items such as the medical history and laboratory findings.

Early analyses of our data contrasting medical and surgical management of coronary artery disease indicated little difference in overall survival but showed reduced incidence of chest pain in the surgically managed group. These results were published in 1974 (2). More recently, the Veterans Administration (VA) randomized study of the coronary vein bypass has found the same result (3). Interestingly, both studies found subgroups of patients in whom one treatment was associated with a better outcome than the other treatment. However, the statistical validity of subgroup inferences is suspect in both our observational study and the randomized trial. It is particularly with these retrospectively identified subgroups that problems of bias can lead to misleading interpretations. Because subgroups are frequently more homogeneous than the original study groups, they are of considerable interest to the practicing physician. With access to data concerning such subgroups, he is able to tailor a management strategy for his patient. In addition, he can subgroup with time as a variable and look for temporal trends in treatment (such as operative mortality). Thus, the level of statistical confidence in inferences based on subgroups derived from randomized and observational studies is of considerable interest.

ASSESSMENT OF SUBGROUP COMPARABILITY

A study based on random allocation of competing interventions has certain advantages at analysis time. One of the strengths of this strategy lies in the accurate estimation of inferential error rates for the hypothesis around which the study was designed (4). However, in search of more specific results, many investigators wish to continue past the original hypotheses, looking for that subgroup in which the outcome differences are either strikingly different or equivalent. This, of course, inevitably leads to problems based on the multiple comparisons. As we have come to appreciate, the more one subgroups, the more likely one is to find unbalanced, outcome-sensitive baseline properties in the (two) comparative groups. Unequal distribution of known covariates across treatment groups can sometimes be dealt with by appropriate models, while unbalance of unknown covariates will inevitably lead to confusion. Such confounding of treatment effects with potential patient effects cannot easily be resolved, and it occurs in observational data bases as well as data developed from randomized trials.

Once one embarks on multiple comparisons outside the design hypothesis, accurate estimation of inferential error rates is lost in a randomized design. Thus, in this setting, a prospective observational study and a random treatment allocation study become equivalent. To illustrate the problems of multiple comparisons in a randomized trial and to investigate further the issues of model-based adjustment of unbalanced baseline attributes, regardless of treatment allocation strategy, we designed a randomized trial of a null intervention, and through multiple comparisons, we retrospectively identified some interesting subgroups in which treatment contrasts were inferentially in error (5).

The subjects in this simulated trial consisted of 1073 medically treated patients with angiographically proven coronary artery disease. The patients were randomized into two groups, designated treatment group 1 and treatment group 2. The design followed that of the VA Cooperative Study. The 1073 patients were stratified according to age (< 50 or ≥ 50) and the number of significantly diseased coronary vessels (one, two, or three). Randomization was applied separately within the six prognostic strata.

As expected, the life-table survival rates of groups 1 and 2 were similar: 89.7% versus 89.2% at 1 year, 76.9% versus 79.1% at 3 years, and 69.3% versus 72.0% at 5 years. Following this, the overall study group was stratified by the number of diseased vessels (one, two, or three) and left ventricular (LV) contraction pattern (normal or abnormal). One of these groups (three-vessel disease, abnormal LV contraction pattern) demonstrated a significant difference in survival between treatments 1 and 2 ($P < 0.025$). This group was composed of 397 patients: 194 receiving treatment 1 and 203 receiving treatment 2. The baseline variables were mildly unbalanced between the two treatments, suggesting slightly severer disease in the group receiving treatment 1.

Table 1 indicates the prevalence of significant baseline factors in the two groups. Note that when testing equivalence of proportions with a chi-square test, we find no significant differences at the 0.05 level among these variables, although LV diffuse abnormal contraction is different at the 0.08 level.

A more important assessment of baseline comparability is based on the joint relationship between these factors as they relate to outcome (survival), and for this purpose we have adopted the survival

TABLE 1.—Comparison of baseline characteristics in subgroup with abnormal LV contraction and 3-vessel disease

Factor*	Prevalence		
	Group 1 (n = 194)	Group 2 (n = 203)	P value
History of CHF	26	24	0.70
Cardiomegaly	33	33	0.99
Resting ST-T wave abnormalities on ECG	67	58	0.23
Significant mitral insufficiency	13	18	0.14
AVO2D > 5.5 vol%	31	24	0.13
LV diffuse abnormal contraction	32	24	0.08
Left main stenosis ≥ 50%	20	19	0.81

*CHF = congestive heart failure; AVO2D = arteriovenous oxygen difference.

TABLE 2.—Comparison of chi-square values for individual and joint prognostic significance of baseline variables

Factor	Chi-square values	
	Individual significance	Joint significance
Treatment	5.4	2.4
History of CHF	36.1	3.8
Cardiomegaly	15.7	0.3
Resting ST-T wave abnormalities on ECG	8.4	2.2
Significant mitral insufficiency	25.1	2.7
AVO2D > 5.5 vol%	52.0	11.1
LV diffuse abnormal contraction	17.3	0.3
Left main stenosis ≥ 50%	6.9	5.7

model of Cox (6). Survival modeling provides not only a means for representing multivariable relationships but also a means for refining the properties of a subgroup by incorporating attributes not included in the subgroup definition, hopefully making "unlikes" more "like" each other. Table 2 indicates the adjusted prognostic significance resulting from this model. The entry in the middle column shows the chi-square statistic whose magnitude indicates relative prognostic significance of each variable considered individually as it relates to survival. The critical value of chi-square used here to reject the null hypothesis is 3.84. On a univariate basis, the "treatment" appears to be a significant factor in the survival of this subgroup (chi-square = 5.4; $P < 0.025$). When the variables are considered jointly, the treatment effect becomes nonsignificant (chi-square = 2.4). Similar results were obtained using the Mantel-Haenszel test for survivorship data (7). In other words, multivariate assessment of survival demonstrated no treatment effect, while univariate assessment of survival demonstrated, for some variables (including treatment), a striking relationship with survival.

An example of the ability of the Cox model to adjust for baseline differences which might be created by the nonrandomized selection of therapy is also illustrated in our series of medically treated patients. This example involved nonrandomly creating two groups of medical patients with different median ejection fractions. We put all patients with an ejection fraction < 40 into one group, all patients with an ejection fraction > 60 into a second group, and randomly allocated the patients with ejection fractions of 40–60 to one of the two groups. The resulting distribution of patients produced one group with a median ejection fraction of 39 and another group with a median ejection fraction of 60. The survival of these two groups differed rather dramatically (chi-square = 45; $P < 0.00001$). Using baseline characteristics other than ejection fraction, we were able to demonstrate with the Cox model that the differences in survival were not due to the group that the patients were in but rather to an inequality of these other baseline characteristics. That is, the group comparison became statistically insignificant when adjustment was made for baseline variables other than ejection fraction. Because these groups were nonrandomly formed so that the large maldistribution of ejection fraction would affect outcome, and because all patients were treated medically, we were reassured when the model was able to tell us that the difference in group outcomes was accounted for by unequally distributed baseline characteristics. Especially interesting was the fact that the significance of the difference could be explained by variables other than the one (ejection fraction) used in forming the two groups.

DISCUSSION

Improving the management of chronically ill patients requires numerous tools, among which we include a repository of accurate clinical experiences, appropriate analytic methods, and effective means for disseminating results of these methods to the practicing physician. Access to a large data base of clinical experiences and accurate models relating outcomes to prognostically significant attributes that characterize past experiences are two such tools we find effective.

The age-old question remains of how best to study biologic systems. The conventional wisdom is to build a rigid box within which treatment strategies are evaluated. A key component of such a strategy is the random allocation of treatment among subjects, with the resulting outcome data then applied to improving the lot of all patients.

The design of randomized studies in the setting of chronic illness is quite broad, and it frequently ignores temporal variation of the disease and the patient environment. Thus, for the practicing physician, the overall findings from such a study have only general applicability to today's patient.

Not content to simply practice average medicine when given access to a repository of patient experiences, the physician and clinical investigator try to identify subsets of the originally studied group that demonstrate a gradient in outcome relative to treatment. We are led to believe that this is "bad" when coupled to an observational study while "acceptable" in a randomized trial. The point is that attempts to sharpen the focus on meaningful subsets, regardless of treatment allocation strategy, lead to difficulties arising from the multiple comparisons.

The more comparisons one makes or the more subgroups one reviews, the greater the likelihood of detecting treatment differences that are associated with unbalanced covariates such as age, sex, or extent of disease. The best analogy to this is that of sampling from all possible subgroups without replacement. Sooner or later one runs low on frequently occurring subgroups that are balanced with respect to prognostically significant covariates and must then select from increasingly more prevalent unbalanced subgroups.

We have thus found that regardless of the mechanism of treatment allocations, the careful investigator is faced with determining when inferential errors are made in assessing treatment effects within subgroups. Randomized clinical trials with fixed data collection periods are not well-suited for dealing adequately with either the multiple comparison problem or changes in technology. Unless data are collected as part of routine care, the costs involved in extending the clinical trial are high and the numbers of available patients are inadequate for assessing inferential errors. The resulting limited number of patients serves in the end to limit the practicing physician's interest in the results. In fact, limiting the size of a study can be seen as contrary to the need for dealing with individualized patient care.

An observational data base with prospective data collection, active followup, and in-line data collection can provide the large numbers of patients required to attenuate problems associated with multiple comparisons. For instance, patients can be randomly partitioned into groups to repetitively test associations. Comparable findings in these independent groups would, of course, strengthen the confidence in the observed comparison. In addition, treatment comparisons can be refined further by using analytic models relating outcome events to baseline attributes. These models transcend individual subgroups through functional approximation and can provide robust estimates of outcome likelihoods through increased numbers. As shown here, a carefully constructed multivariable model can be used to explain perceived treatment differences. However, the large number of uncontrolled covarying attributes requires large numbers of patients for robust parameter estimation.

Longitudinal data bases are required to properly assess temporal variation in attributes of prognostic importance. For instance, high operative mortality during the early years of a study can mask treatment effects while the surgical team develops its operative skill and evolves patient selection criteria. Later, as operative mortality drops, treatment effects may be masked if early data are included. Only by collecting data continuously can such temporal shifts be detected. In addition, as new interventions become available, early results can be compared with results from a large control group extracted from earlier studies within the data base. Such use of historical controls has been demonstrated quite successfully by Gehan and Freireich (8). Here the temporally varying attribute of interest is the treatment itself. Finally, with longitudinal data, we can increase our confidence in an early identified subgroup demonstrating an outcome gradient referenced to treatment if the results are repeatable with recent members of the subgroup.

Clearly, observational studies derived from prospective data collection cannot be treated casually. The trap of making inferential errors is real, but randomized treatment allocation cannot drive the error rate to zero. The advantages of a prospective data bank, managed in line with routine patient care, are on the side of chronic illness. The costs can be shared with patient care, thereby providing a mechanism for collecting long-term data on large numbers of patients that demonstrate the dynamics of the illness, the patient, and the surrounding environment. As we have described, the resulting observational data base can be used to identify prognostically important variables; detect temporal shifts in patients, disease, and technology; and provide data on subgroups that may aid the practicing physician. In addition, the data base can serve as a precursor to a randomized trial by defining those subgroups most likely to respond to a new or unproven treatment.

Inferential errors resulting from multiple comparisons are found in both observational and randomized designs. Multivariable models can aid in adjusting for covariate effects, thus reducing the likelihood of patient-related effects confounded with treatment. In the final analysis, though, real increases in confidence in treatment effects require independent confirmation by other investigative groups.

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Randomized Clinical Trials and Research Strategy[1]

Richard Simon[2,*]

SUMMARY

This paper attempts to clarify the relationship between randomized treatment assignment and the basis for statistical inference. The relationship between research strategy and study size is also discussed. The need for confirmatory studies is emphasized. Comments concerning postrandomized consent designs are also presented.

[Cancer Treat Rep 66:1083–1087, 1982]

Good clinical therapeutic research identifies useful treatments, provides reliable leads, or appropriately categorizes useless or harmful treatments. Effective clinical research requires good methods and good strategy. This presentation will attempt to accomplish three goals: first, to outline some of the advantages of randomized treatment assignment as a research method; second, to briefly comment on some of the alternatives; and third, to describe a framework for viewing research methods as part of a general research strategy.

There have been many previous debates and discussions about the importance of randomization for comparative studies. It is not the intent of this paper to review or repeat all of the arguments that have been made. Instead, I will attempt to clarify the relationships between treatment assignment mechanism, basis for statistical statements, and research strategy.

RANDOMIZED TREATMENT ASSIGNMENT AND STATISTICAL INFERENCE

Stratified randomization will be included in my use of the term "randomization," and I will assume that two treatments are being compared. There are two basic statistical approaches for drawing conclusions about relative therapeutic efficacy from data. The first approach entails no assumptions that the patients studied are a random sample from a larger population if randomized treatment assignment is used. Since the patients receiving treatment 1 are a random sample of the patients entered in the trial, one can calculate the probability of obtaining an assignment of treatments to patients that results in a difference in average outcomes (eg, treatment group response rates) at least as large as actually obtained. This probability is calculated by assuming that the two treatments are equivalent with regard to effect on outcome for each patient, and it thus represents a significance level. Many commonly used statistical significance tests can be viewed in this manner, even tests that adjust for known prognostic factors (1).

The second approach to statistical inference involves an assumption that the patients studied can be viewed as a random sample of some larger population of patients. Though we rarely if ever randomly sample patients, it is generally reasonable to assume that there is *some* target population from which our patients can be viewed as a random sample. The target population is not all patients with the disease in question because of explicit eligibility criteria in therapeutic protocols and because of subtle aspects of referral patterns. Randomized treatment assignment ensures that the target population is the same for both treatment groups.

The use of random treatment assignment together with the assumption that the study patients can be viewed as a random sample of some target population ensures the following. (*a*) The estimate

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[1] Received Sept 2, 1981; accepted Sept 11, 1981.

[2] Biometric Research Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* Richard Simon, PhD, Biometric Research Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 3B16, Bethesda, MD 20205.

of average relative therapeutic efficacy approaches the true relative efficacy for the target population as the sample size increases. In statistical terms, the estimator of relative efficacy is "consistent." (*b*) For finite sample sizes the estimator of relative efficacy is "unbiased"; its expected value is neither larger nor smaller than the true value for the target population.

Randomized treatment assignment does not ensure "comparability" of the treatment groups with regard to known prognostic factors and it does not justify ignoring evidence of serious imbalance. Royall (2) has named such a rigid view of randomization the "closurization principle": "First you randomize, then you close your eyes." Randomization is also not necessary to achieve balance on known prognostic factors. This fact has often been used spuriously by opponents of randomization. The main purpose of randomization is not for controlling known prognostic factors but rather for statistically controlling unknown factors affecting outcome.

This statistical control is accomplished in the following way. Randomization ensures that the totality of unknown and uncontrollable sources of variability will have a known symmetric distribution between the treatment groups. This permits us to make statements about the statistical properties of our estimators. For example, we can calculate confidence intervals for the true treatment difference and significance levels. Thus, in addition to an unbiased and consistent point estimate, we can obtain a probabilistic quantitation of the precision of our estimate or a measure of the credibility of hypotheses. Without randomization we obtain confidence limits and significance levels only by *assuming* a random and symmetric distribution for uncontrollable sources of variability.

The potential sources of bias for nonrandomized studies have been previously discussed (3–6). They range from overt differences in selecting patients for treatment to subtle changes in referral patterns and developments in diagnostic and staging technology. Whether a difference in average outcomes for nonrandomized treatment groups is therapeutically meaningful with regard to any target population can be assessed only by subjective evaluation of the possible sources of bias. Such an evaluation rarely can go beyond examining comparability with regard to known prognostic factors. Consequently, the conclusions are inherently more suspect than those for a randomized study. For many nonrandomized studies there are obvious differences between the treatment groups with regard to known prognostic variables. These differences can be accommodated by matching or using regression models. But the presence of such differences makes very questionable the assumption of randomness for the unknown sources of variability.

HISTORICAL CONTROLS

Almost everyone would agree that historical controls are adequate in evaluating a new treatment for a uniformly and rapidly fatal disease. Even in less extreme circumstances, historical controls have been adequate for identifying major therapeutic improvements. Unfortunately, useless or harmful treatments and false leads are more frequent than major therapeutic advances, and it is very difficult to distinguish the two based on nonrandomized studies. Also, most clinical trials in oncology are characterized by considerable heterogeneity in the patients treated and by variability in the outcomes obtained. Consequently, the opportunity for bias in nonrandomized studies is generally considerable.

Most studies using literature controls or old historical controls suffer from the absence of any substantial and scientifically skeptical attempt to examine sources of bias that may affect the results. Gehan and Freireich (7) and Pocock (8) have given serious attention to conditions under which historical controls may serve as a reliable basis for comparison. Pocock's conditions are essentially as follows. (*a*) The control group must have received a precisely defined control treatment in a recent previous study performed in the same institution(s) with largely the same clinical investigators. (*b*) Requirements for patient eligibility, workup, and outcome evaluation must be the same. (*c*) Important prognostic characteristics should be known and be the same for both treatment groups. (*d*) There must be no other indications leading one to expect different results, eg, more rapid accrual in the new study.

The approach of Gehan and Freireich (7) places greater emphasis on identification of the major prognostic variables and uses regression methods to account for their effect.

Though many prognostic factor analyses are published, the unexplained sources of variability are generally much more important than the factors identified. The greatest effect found in the Coronary Drug Project was the positive benefit of good treatment compliance for the placebo group (9). The 1813 patients who took at least 80% of their placebo had a 5-year mortality rate of 15.1%. The remaining 882 patients given placebos had a 5-year mortality rate of 28.2%. The significance level for this comparison was 4.7×10^{-16}. Since this difference must be due to imbalances in prognostic variables, the investigators sought to account for it by a multiple regression analysis including > 40 baseline factors. Attempts to adjust away the difference only succeeded in increasing the significance level to 7.4×10^{-9}. Clearly, major prognostic determinants were not contained in the known or suspected variables.

Pocock (10) has reported 19 unselected cases in which the same treatment was used in consecutive trials of the same cancer cooperative group. In four of the 19 cases (21%), the difference in outcomes was statistically significant at the 0.02 level (10). Farewell and D'Angio (11) have shown similar results for the national Wilms' tumor studies. They were not able to account for the difference by adjusting for potential prognostic factors using the proportional hazard model. They also document that for their study, a 2/1 randomization would not have given a sufficient number of concurrent controls to notice the problem.

We tend to overestimate our knowledge of prognostic factors. Large prognostic factor studies are very important and should be vigorously pursued. When prognostic factors are really well-understood, reliable randomized studies will require very few patients.

DATA BANKS

Computer files of clinical data collected under nonexperimental conditions have been used for many years for a variety of purposes. Useful studies of prognostic factors and the natural history of diseases have been accomplished. Population-based tumor registries provide valuable information for monitoring the incidence of and mortality from cancers. There have also been brilliant successes of nonexperimental studies in epidemiology. Epidemiologists rarely have a choice of experimental versus "observational" approach. Accounts of the classical triumphs of epidemiology show the work of exceptional individuals with great tenacity, thoroughness, and scientific skepticism.

Computer files of clinical data collected under poorly controlled conditions are, however, ill-suited for reliably comparing treatments. Historical control studies based on series of consecutive patients at least attempt to define the population under study and to standardize treatment, workup, and response evaluation. With "data bank" studies the possibilities for bias in treatment assignment are much greater, and there may be major problems with missing or inaccurate data, resulting from lack of standardization. Comparing poorly defined treatments for numerous poorly defined subsets of patients also leads to insurmountable problems of multiplicity (12). Other major problems are described by Byar (13) and by Dambrosia and Ellenberg (14). Though data banks may serve useful purposes as information retrieval mechanisms for planning experimental studies, they are not generally a reliable basis for resolving important questions of public welfare.

POSTRANDOMIZED CONSENT

If no patients refuse the treatment offered them in a postrandomized consent design, then all the usual advantages of randomization are preserved. As in the conventional randomized design, patient refusals cause problems in the interpretation of results. It is generally accepted by statisticians that exclusions after randomization should be avoided, because they can introduce bias (15). To the extent that the postrandomized consent design is associated with an increased number of patients refusing the treatment, the problems of analysis and interpretation will be more severe. Zelen (16) has shown for a postrandomized consent design how to perform a statistical significance test comparing the policies of trying to administer the treatments under study. He has also tabulated the required increase in sample size as a function of the patient refusal frequency. If there are many refusals, however, results may be misleading.

To take a hypothetical example, suppose that old patients tend to refuse an intensive therapy. Suppose also that the two treatments are equivalent on the average for mixed groups of patients but that the intensive therapy prolongs survival for young patients while shortening survival for older ones. Under these conditions, a significance test comparing the treatment groups as randomized will indicate that the intensive treatment policy is preferable for the mixed sample. This would be an inappropriate conclusion and proper interpretation of results may be very difficult to determine.

These potential problems also apply to conventionally randomized studies. But in the latter, one tries to eliminate exclusions by randomizing only patients who agree to accept either treatment. Postrandomized consent designs are being usefully employed in oncology today. I believe, however, they should be introduced only in situations where the conventional practice of seeking consent before randomization has proven troublesome. For studies employing postrandomized consent, the patient refusal rate should be closely monitored.

THERMODYNAMICS OF CLINICAL TRIALS

As stated in the introduction, effective therapeutic clinical research requires good strategy as well as good methods. In this final section, one aspect

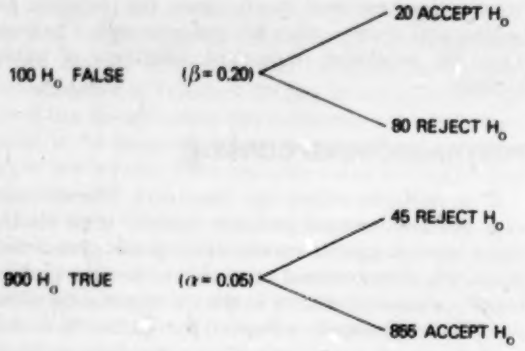

FIGURE 1.—Results from 1000 hypothetical clinical trials.

of the relationship between the two concepts will be described. I have called this "thermodynamics" because it concerns aggregate results from a program of clinical research.

Figure 1 depicts "results" from 1000 hypothetical clinical trials. It is assumed that in 100 cases the two treatments have different effects on prognosis and in 900 cases they are identical. Each outcome is simplistically categorized as to whether the observed treatment difference is statistically significant at the 0.05 level (reject H_0) or not. Each clinical trial is assumed to be of a size such that the power for detecting a specified difference (the true difference in cases where H_0 is false) is 0.80. With this oversimplified framework, one obtains the expected frequencies shown at the right of the figure. Under the assumed conditions, there is in fact no real difference in 45 (36%) of the 125 cases where we claim a statistically significant ($P < 0.05$) difference between the treatments.

Staquet et al (17) have called this 36% figure the δ error rate. It is the expected number of false-positive trials divided by the expected number of positive trials. It can be written as

$$\delta = \frac{(1-\pi)\alpha}{(1-\pi)\alpha + \pi(1-\beta)}$$

where α is the type 1 error rate for the set of trials (eg, $\alpha = 0.05$), $(1-\beta)$ is the power of each trial for detecting an important true difference (if it exists), and π is the proportion of the trials for which a clinically important true difference exists.

Staquet et al also define the ε error rate as the expected number of false-negative trials divided by the expected number of negative trials:

$$\varepsilon = \frac{\pi\beta}{\pi\beta + (1-\pi)(1-\alpha)} .$$

TABLE 1.—δ and ε error frequencies for aggregates of clinical trials*

α	β	π	δ	ε
0.05	0.20	0.01	86%	0.2%
0.05	0.20	0.10	36%	2.3%
0.05	0.20	0.20	20%	5.0%
0.01	0.20	0.10	10%	2.2%

*α and β represent the type 1 and type 2 error frequencies for the set of clinical trials. π is the proportion of trials in which one treatment is truly superior to a clinically meaningful extent. δ is the expected % of positive results that are false-positives. ε is the expected % of negative results that are false-negatives.

The quantity π is of course unknown, but Staquet et al believe that for comparative trials in oncology it is < 20%. Table 1 shows the δ and ε error rates that result from assumed values for α, β, and π. With a small assumed value for π, this analysis implies that reasonably small values of δ are obtained only by using a type 1 error rate (α) on the order of 0.01 or less, rather than 0.05. If the power $(1-\beta)$ of the studies for detecting a difference in therapeutic efficacy of fixed magnitude is to be maintained, the number of patients included must be increased. For example, to detect a change in complete response rate from 20% to 40% with $\beta = 0.20$ and $\alpha = 0.05$ (two-sided) requires approximately 80 patients per treatment. Using $\alpha = 0.01$ increases the required sample size to about 120 patients per treatment.

This analysis and similar ones by Zelen et al (18) and Peto et al (15) clearly demonstrate that positive findings from small or moderate-size clinical trials, whether well-done using the best methodology or not, require confirmation by others. Independent confirmation is a widely accepted scientific principle, but the need for it is often not sufficiently appreciated in the discussion section of published clinical papers.

From the research strategy point of view, this analysis does not necessarily imply that only large randomized clinical trials should be performed. There are strong advantages to conducting moderate-size innovative trials with the intent of reconfirming those that are positive by doing larger trials. In addition to encouraging innovation, this approach devotes large studies only to treatments that are promising in moderate-size studies.

The δ and ε error rates can be calculated for a two-stage approach in which a treatment is considered "positive" if it passes both stages of testing and in which the second-stage confirmation study is performed only for treatments found promising in the first stage. These error rates are

$$\delta = \frac{(1-\pi)\alpha_1\alpha_2}{(1-\pi)\alpha_1\alpha_2 + \pi(1-\beta_1)(1-\beta_2)}$$

$$\varepsilon = \frac{\pi[\beta_1 + (1-\beta_1)\beta_2]}{\pi[\beta_1 + (1-\beta_1)\beta_2] + (1-\pi)[(1-\alpha_1) + \alpha_1(1-\alpha_2)]}$$

where $(\alpha_1, 1-\beta_1)$ and $(\alpha_2, 1-\beta_2)$ are the nominal type 1 error rates and powers for the two stages, respectively. Simple calculations show that the first-stage trial need not be large in order to keep the δ error rate small. The greater problem is that a lack of power at the first stage cannot be remedied at the second stage, because the second stage will not be reached. From this perspective, reasonable designs would have first-stage cutoff significance levels (α_1) in excess of 0.05, say 0.10. The parameter α_1 cannot be increased too much without passing numerous spuriously promising treatments into the larger second-stage design. It must be recognized that many trials that pass the first stage represent spurious treatment differences.

Historical control studies performed as described by Pocock (8) are less objectionable if they are viewed as stage one screening trials. Since the significance levels for historical control studies do not account for systematic (nonrandom) differences, the resultant effect is to increase α_1.

For fields not characterized by numerous studies, the above considerations are not relevant. Even for oncology there are problems. These are primarily the reluctance of investigators to recognize that positive results require confirmation and sometimes the difficulty of conducting a confirmatory study in light of impressive initial results (19). The two-stage approach is similar to what is actually practiced in oncology today, except that an $\alpha_1 = 0.05$ is considered inviolate. This discussion has not been for the purpose of introducing a new approach. Rather, the purpose has been to clarify some advantages of current research strategy, to provide a perspective for the role of diversity in study design, and to reemphasize the need for confirmation and cautious interpretation of results.

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Design of Controlled Clinical Trials: Use of Historical Controls[1]

Edmund A. Gehan[2,*]

The following brief review of some actual clinical trials leads to the conclusion that historical controls can often be utilized for evaluating the relative effectiveness of therapies. Certainly, not all historical control groups are of value in this regard; however, careful analysis, with special attention to adjusting for relevant prognostic features and sensitivity to possible biases in the evaluation of results, can lead to inferences about therapies.

The examples to be presented are illustrative of the concepts that: (*a*) nonrandomized studies can result in evaluation of the relative effectiveness of therapies, both for control and experimental treatments; and (*b*) randomized studies can be evaluated for nonrandomized elements in them, both for other treatments that may have been administered at some institutions and for subgroups of patients that may demonstrate differential effectiveness of treatments. Various studies in cancer will be considered.

Farewell and D'Angio (1) have recently given a simulated example of consecutive studies in Wilms' tumor wherein a new therapy could be declared not more effective or more effective than control treatment depending on whether a historical or concurrent control group was utilized. Chalmers et al (2) have given examples demonstrating that historically controlled studies generally indicated anticoagulant therapy to be of value following myocardial infarction, whereas concurrently controlled studies did not.

STUDY OF 6-AZAURACIL IN ACUTE LEUKEMIA

The report of this clinical trial appeared in 1960 (3) and began as follows: "Although leukemia is at present a uniformly fatal illness, its clinical course is quite variable. Complete and partial remissions of the disease may occur spontaneously as well as in response to chemotherapeutic agents." After administration of what then were the only active chemotherapeutic agents (folic acid antagonists, adrenocorticosteroids, and purine analogs), no specific therapy was available. Hence, new agents were studied, with no available data concerning the natural history of the refractory phase of the disease.

The study was designed as a randomized double-blind comparison of 6-azauracil versus placebo in patients in the relapse phase of acute leukemia. Table 1 shows that there were 28 patients in the randomized comparative study and 23 patients who received 6-azauracil in a study that was nonrandomized. There was objective improvement in about 30% of the patients in both the randomized and nonrandomized studies, with no evident difference between 6-azauracil-treated and control patients. Survival curves for each of the three groups of patients indicated a median survival time of approximately 3 months from the start of the study to death.

In the conclusions, 6-azauracil was found to have "no significant antileukemic activity"; however, the study was of importance in defining the natural history of the advanced refractory phase of acute leukemia. There was evidence that the noncontrolled study group acted in a manner similar to that of the comparative study group. This study was a forerunner to some later cooperative studies in which there was no double-blind randomized comparison group but only a noncontrolled study group.

PROTECTED ENVIRONMENT PROGRAM IN THE CHEMOTHERAPY OF ACUTE LEUKEMIA

Two examples of clinical trials evaluating the protected environment (PE) in the treatment of acute leukemia patients will be given: the first (4), nonrandomized, indicated the effectiveness of the PE, and the second (5), randomized, confirmed the earlier results. The nonrandomized study had no formal statistical design. That is, 33 patients with acute leukemia underwent chemotherapy while in a PE and received prophylactic antibiotic regimens. No plan was made for a control group, randomized or otherwise. Two control groups of 33 patients were selected from patients treated at approximately the same time to be as comparable as possible to the

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[1] Received Sept 18, 1981; accepted Sept 18, 1981.

[2] Department of Biomathematics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Edmund A. Gehan, PhD, Department of Biomathematics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Summary of hematologic responses observed in randomized and nonrandomized study of 6-azauracil

	No. of patients					
Phase of study	Total	Complete remission	Partial remission	Hematologic improvement	Reduction of circulating leukemic cells	Total objective improvement (%)
Comparative study groups						
6-Azauracil	15	0	0	2	2	27
Control	13	0	1	1	2	31
Total	28	0	1	3	4	29
Noncontrol study group	23	1	1	3	2	30
Total	51	1	2	6	6	30

TABLE 2.—Characteristics of potential pairmates and PE patient at the start of treatment*

Patient	Study	Diagnosis	Time from diagnosis to therapy (wks)	Previous therapy	Sex/age	Infected	Wbc count† (% blast cells)	Platelet count†	Clinician rank‡	PE or C
1	POMP	ALL	2	None	F/20	No	19 (69)	315	1	
2	POMP	AML	1	None	M/20	No	10 (93)	43	3	
3	POMP	AML	15	Pred,Mtx, 6MP	M/16	Yes	16 (67)	66	4	PE
4	POMP	ALL	1	None	M/17	No	35 (79)	43	2	
5	POMP	AML	102	6MP	M/18	Yes	38 (88)	28	4	C_1
6	POMP	AML	0	None	M/18	No	28 (71)	42	3	C_2

*POMP = 6MP, vincristine, Mtx, and Pred; ALL = acute lymphocytic leukemia; AML = acute myelocytic leukemia; Pred = prednisone; Mtx = methotrexate; 6MP = 6-mercaptopurine; and C = control.

†Cells × 10^3/mm^3.

‡Patient ranked 1 had best prognosis, 2 next best, etc.

PE group with respect to chemotherapy regimen, diagnosis, age, sex, infection status, wbc count, platelet count, time from diagnosis to therapy, and previous therapy. Table 2 gives a list of the potential pairmates for one of the PE patients, indicated as the third patient in the table. This designation was not known to Dr. Bodey, who ranked the six patients according to his judgment of the probability of response. Note that the PE patient received a rank of 4 and the two control patients selected had ranks of 4 and 3, respectively. Control patients were selected to have equivalent or superior ranks to those for the PE patients. Two pairmates were selected for each of the 33 patients in the PE in the same manner, and the differences in response rates, infection rates, length of response, and survival were analyzed, comparing PE with control patients and comparing patients within each pair of controls.

The complete remission rate was 61% for the 33 PE patients and 49% for the 66 patients in the combined control groups. The difference of 12% was not near statistical significance but was suggestive. There were significantly fewer episodes of both local and severe infection as related to neutrophil count in the PE compared with control patients. For example, the episodes of local infection per 1000 days at risk were 27.9 and 11.0 for PE patients with neutrophil counts of < 100/mm^3 and 100–999/mm^3, respectively. The incidence was 54.1 and 16.8, respectively, in the combined control group. For severe infections, the incidence was 15.0 and 7.6 episodes per 1000 days at risk for PE patients according to increasing levels of neutrophil count, compared with 20.5 and 15.8, respectively, for patients in the combined control groups. There was a significant improvement in survival for PE patients, the median survival being about 40 weeks for PE patients compared to 22 weeks for the combined control patients.

A justification for comparing PE versus control was the comparisons between the two control groups of 33 patients to test the process of selection. No differences in complete remission rate, incidence of infection, or survival were demonstrated in a paired analysis between these groups.

A conclusion of the paper was: "The use of nonrandomized patients as a control group has been considered unacceptable by some investigators.

However, this approach was both necessary and advantageous for our study. Randomizing patients to either the PE group or a control group would have resulted in the PE units being unoccupied for substantial periods of time and would have required a much longer time to complete the study. Randomization would not have guaranteed that patients in the PE group and the control groups would be comparable, whereas our selection procedure ensured that the pairmates were as comparable as possible with respect to nine patient characteristics which have been demonstrated to affect prognosis." This paper, published in 1971, was early evidence in cancer clinical trials that a nonrandomized study could lead to conclusions about treatments.

A randomized study of a PE-prophylactic antibiotic program was subsequently carried out in patients with acute leukemia. The design of the study is given in table 3; it was analyzed as a 2^2 factorial design, the two factors being PE unit status (yes or no) and method of antibiotic administration (oral or iv).

The results can be summarized as follows: the complete remission rate was 71% in 63 PE patients compared with 43% in 82 non-PE patients, a highly significant statistical difference ($P < 0.01$); the median survival time was about 80 weeks for PE patients compared with 50 weeks for non-PE patients, again a highly significant difference ($P < 0.01$); the incidence of infectious episodes according to neutrophil count was significantly lower for PE patients.

Hence, this is an example of a treatment, PE, that was first detected as beneficial in a nonrandomized study and subsequently confirmed in a randomized one.

EVALUATION OF MITHRAMYCIN IN THE TREATMENT OF ANAPLASTIC GLIOMAS

This is an example of a randomized prospective clinical trial in which (*a*) all patients underwent surgery and (*b*) the treatment under study, mithramycin, was not effective in enhancing survival relative to no treatment (6). However, there was strong evidence that radiotherapy, which was administered at the discretion of the investigators, did influence survival.

From a total of 116 patients entered in the study, 96 were in the valid study group; 52 underwent surgery and received mithramycin at a dose of 25 μg/kg/day for 21 days, and 44 had surgery only.

Patients in the two groups were comparable with respect to age distribution, sex, location of tumor, diagnosis, tumor characteristics, signs or symptoms, and radiotherapy received. There was no evidence of a difference in survival between the two treatment groups; the median survival time for patients receiving mithramycin was 21 weeks, whereas it was 26 weeks for those receiving conventional care (surgery plus no further treatment).

TABLE 3.—Randomization of patients in PE study*

Randomization	No. of patients
PE unit available	
PE, PA	29
PE, PA	34
SA_1	29
No PE unit available	
SA_2	27
PA	26

*PA = oral antibiotics and SA = systemic antibiotics.

Radiation therapy was not randomized in the study; however, 48% of the patients in both the mithramycin and control groups received $\geq$ 3000 rads. Since the percentages of patients receiving radiotherapy were comparable, a survival comparison was made between patients who did or did not receive the radiation therapy. The median survival time for patients who received some radiation therapy was 36 weeks compared to 15 weeks for patients who did not receive radiotherapy; the difference between survival curves was statistically significant ($P < 0.05$).

Adjustments were made for both the number of doses of mithramycin received and the dosage of radiotherapy, and comparisons were made between mithramycin and control groups. Table 4 shows that there was effectively no relationship between increasing number of doses of mithramycin and survival. However, median survival time tended to increase according to the number of rads received at each number of mithramycin doses (none, < 16, ≥ 16).

The number of patients in some of these groups was quite small, and unknown factors may have made patients who received radiotherapy more favorable than those who did not. Further factors that were controlled for in comparing radiotherapy and no radiotherapy were length of time required to receive the radiotherapy and duration of symptoms. After adjustment for these factors, the essential conclusion remained the same: patients receiving radiotherapy tended to survive longer than those not receiving it. The conclusion of the paper stated: "Although the study was not designed to evaluate the efficacy of radiotherapy, patients who were so treated had a significant improvement in survival." The radiotherapy findings were the most notable aspect of the study and they have subsequently been confirmed in a randomized study.

TABLE 4.—Relationship of dosage of mithramycin and irradiation to survival time

No. of doses of mithramycin	X-ray (rads)	No. of patients	Median survival time (wks)
None	None	20	15
None	< 5000	10	33
None	≥ 5000	14	36
< 16	None	17	19
< 16	< 5000	4	3
< 16	≥ 5000	7	33
≥ 16	None	17	19
≥ 16	< 5000	12	38
≥ 16	≥ 5000	8	48

TABLE 5.—Complete remission (CR) rates at the 3 largest institutions vs other institutions, adjusted for predicted probabilities of response

Treatment	No. of patients	% CR observed	% CR expected	% difference	P*
		Three largest institutions			
COAP	27	41	51	−10	0.24
OAP	35	54	54	0	> 0.90
DOAP	28	71	53	+18	0.03
Total	90	56	53	+3	> 0.10
		Other institutions			
COAP	49	37	46	−9	0.16
OAP	42	54	54	0	0.24
DOAP	37	27	39	−12	0.10
Total	128	34	44	−10	0.02

*Statistical significance level for observed vs predicted response rate.

EVALUATION OF COMBINATION CHEMOTHERAPY IN ACUTE LEUKEMIA

In a study conducted by the Southwest Oncology Group (SWOG) (7), a total of 274 patients with AML were randomly assigned to one of three regimens: vincristine, prednisone, and cytarabine (OAP); the same treatment plus 100 mg/m^2 of cyclophosphamide (COAP); or the same treatment plus 100 mg/m^2/day of daunorubicin (DOAP).

In the largest group of patients according to diagnostic type and prior treatment, namely AML patients with no prior treatment, the complete remission rates were 37% for COAP, 35% for DOAP, and 43% for OAP. The differences in complete remission rate were not near statistical significance in this or any other category of patients in the study. Among patients with no prior therapy having received an adequate trial, the complete remission rates were 72% for COAP, 68% for DOAP, and 82% for OAP. The differences among these rates were not near statistical significance. Further, among all previously untreated patients with AML, there was no statistical evidence that survival length differed according to induction treatment ($P > 0.36$).

From a large retrospective study of acute leukemia patients at the M. D. Anderson Hospital and Tumor Institute, a logistic regression equation was calculated in which six prognostic factors were identified as being related to probability of complete remission. These were: type of acute leukemia, age, hemoglobin value, logarithm of platelet count, weeks from symptoms to diagnosis, and logarithm of wbc count. In table 5, a comparison is made between the observed percent and expected percent of complete remissions, using the logistic regression equation for each of the treatments at two groups of institutions: (*a*) the three largest institutions and (*b*) all other institutions. Several points may be noted. First, the predicted complete remission rate of 53% at the three largest institutions was significantly higher than the 44% predicted rate at the other institutions ($P < 0.05$). Second, the observed complete remission rates at the smaller institutions tended to be lower than predicted and, for all three treatments combined, the observed rate was 10% lower ($P < 0.02$). At the three largest institutions, DOAP treatment had a CR rate that was 18% higher than predicted, which was statistically significant ($P = 0.03$).

This table was not in the published paper; however, the clinical study group has conducted further studies using a variant of DOAP treatment. The remission induction treatment in the SWOG and other clinical groups generally consists of doxorubicin (which is very similar to daunorubicin) combined with OAP treatment. There were 18 institutions entering patients in this study. Of the 358 entered and eligible patients, 136 (38%) were entered by the three largest institutions.

Analyses of available prognostic factors were made to try to explain the differences in observed versus predicted response rates at the larger and smaller institutions. No particular factor could be identified to explain the differences. It is possible that daunorubicin tends to be relatively more effective in the patients with more favorable prognoses or that clinicians at the three largest institutions were relatively more familiar with utilizing daunorubicin. In this randomized prospective study, evaluation of subgroups of patients has provided a lead for further studies.

CONCLUSIONS

These examples have suggested that historical control groups can be used for evaluating the relative effectiveness of treatments in cancer clinical studies, if careful analyses are carried out and appropriate adjustments made for the prognostic features of the patients. Evaluations of subgroups of patients using the same principles can provide leads for the conduct of future studies. Confirmation of results obtained is an essential aspect of both randomized and nonrandomized studies.

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Strategy and Alternate Randomized Designs in Cancer Clinical Trials[1,2]

M. Zelen[3,*]

A large number of clinical trials are currently being conducted to find better ways of treating cancer. These trials are quite varied: some are based on small numbers of patients, others on large numbers of patients; some are multi-institutional, others are carried out within a single institution; some are randomized, others are nonrandomized. However, regardless of the scientific quality and the care with which a trial is carried out, if the clinical investigator claims that the therapy under investigation is beneficial, it is likely to influence patient treatment everywhere. The problem of choosing the "best" treatment for the cancer patient is difficult and, for many cancer sites, unresolved. One reason for this is that the outcome depends on many factors; eg, anatomic stage, pathology, demographic factors, physical status of the patient, prior history, extra-disease symptoms, and special characteristics of the natural history of the disease site. One point of universal agreement is that the treatment outcome with most patients is not well-predicted because of the wide fluctuations and complex nature of the natural history of the disease. Poorly conducted trials may ignore the influence of characteristics of the natural history of the disease and report a positive effect of therapy, which may be an artifact.

Advances in surgery and radiotherapy have not radically changed treatment outcomes in cancer. Often the change introduced by modifying surgery is to do less surgery. The idea is that less surgery would achieve the same therapeutic effect as more surgery. However, one is not expecting increased cure rates or longer survival. Replacing a radical mastectomy by a simple mastectomy will not result in a better prognostic outlook for the patient with breast cancer. Similarly, replacing surgery with radiotherapy will not improve prognosis. Since it is widely recognized that most cancers are systemic, the emphasis in therapeutic research is to find chemical therapies that can benefit the patient by systemic treatment. The bulk of the clinical trials are chemotherapy studies or trials in which chemotherapy is combined with surgery and/or radiotherapy. In addition to single agents, these chemotherapy experiments emphasize combinations of agents. The number of possible combinations, dose schedules, and regimens seems endless and has generated thousands of cancer clinical trials.

It is a curious, but not surprising, fact that publications of therapeutic investigations in cancer tend to be dominated by "positive" results. Editors and even authors are reluctant to publish negative results. A journal does not gain in scientific reputation nor does an investigator become acclaimed by publishing negative studies. Hence, the question arises as to how many false-positive studies are likely to be published in the literature. Since the 5% false-positive rate is the objective criterion for deciding if a therapy is beneficial or not, we would expect (on the average) 50 positive results for every 1000 null clinical trials. It is estimated that there may be 6000–10,000 studies currently being carried out. These in turn would ultimately generate 300–500 false-positive reports if all of these studies were, in truth, negative.

Of course, one cannot distinguish a reported false-positive from a true-positive therapy. To fully accept a report of a positive study, one should have a well-conducted confirmatory study. However, such a study would ordinarily take approximately the same length of time as the initial study and thus involves similar resources. Some investigators regard confirmatory studies as "duplicative" or "redundant." Furthermore, there is an ethical problem in dealing with the patient. How does the attending physician advise the patient to enter a study in which there is a real possibility that a reported beneficial therapy would be withheld? As a result, confirmatory studies are rare. We may now have reached an impasse in cancer chemotherapy in which there are so many false-positive therapies in the clinic that they are overwhelming the true-positives. Many physicians have a deep feeling that very few pa-

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[1] Received Feb 5, 1982; accepted Feb 15, 1982.

[2] Supported in part by Public Health Service grants CA-06516 and CA-23415 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Sidney Farber Cancer Institute and Harvard School of Public Health, Boston, MA

Reprint requests to: Marvin Zelen, PhD, Department of Biostatistics, School of Public Health, Rm 706, 677 Huntington Ave, Boston, MA 02115.

tients may be benefitting in any significant way from most of the treatments being administered. Who can strongly defend the current treatments for lung and colorectal cancers? Do women receiving adjuvant chemotherapy for breast cancer actually live longer, or do they simply have a longer disease-free period?

STRATEGY AND TACTICS

We shall examine the strategy and tactics of the clinical trial research process. To examine the process, we require three elements that underlie the entire phenomenon. These are the prior probability of success (denoted by θ), the true-positive rate (denoted by β), and the false-positive rate (denoted by α).

The prior probability of success represents the expectation before the trial starts that the result will be positive. It reflects the level of clinical innovation and basic science that go into the decision to initiate a trial. If ineffective drugs are simply grouped into a new combination, then the prior expectation of success may be low. Why should combining ineffectual treatments result in a positive therapeutic advance? On the other hand, a trial based on promising pilot studies or new findings from the laboratory may have a much higher prior expectation of success.

The true-positive rate (the probability of finding an effective treatment, if it is in truth effective) is dependent on both the number of patients in a trial and the value of the false-positive rate. Increasing either will result in an increase of the true-positive rate.

Finally, by universal scientific agreement, most investigators choose a false-positive rate of 5%. Lowering it to 1% *decreases* the true-positive rate. Increasing it to a higher value may result in too many false-positives.

Suppose we had 1000 clinical trials and adopted values for θ, β, and α of 0.10, 0.30, and 0.05, respectively. Then 10% or 100 trials will be true-positives; among these true-positives only 30% or 30 will be reported as positive. Among the 900 true-negative studies, 5% or 45 will be reported as positive studies with the remainder being reported as negative. Thus, the ratio of the number of false-positives to true-positives is

$$R = \frac{\text{No. of false-positive results}}{\text{No. of true-positive results}} = \frac{45}{30} = \frac{3}{2}.$$

Consequently, for every two true-positive therapies, there will be three false-positives. Another way of viewing these results is that the *probability of a reported positive result being a false-positive is 60%*. Even if all 100 true-positive values were reported to be positive, the ratio of false-positives to true-positives would be

$$R = \frac{45}{100} = 0.45$$

and the *probability of a reported positive result being a false-positive is 31%*. In other words, varying the true-positive rate from 30% to 100% still results in a relatively high number of false-positive therapies being used in the clinic.

It is easy to write an explicit formula for the ratio of false-positives to true-positives in terms of θ, β, and α. This formula is

$$R = \frac{\text{No. of false-positive therapies}}{\text{No. of true-positive therapies}} = \frac{(1-\theta)\alpha}{\theta\beta}.$$

The formula for the probability of a reported positive being a false-positive is

$$P = \frac{(1-\theta)\alpha}{(1-\theta)\alpha + \theta\beta} = \frac{R}{1+R}.$$

The above numerical example is based on the parameters $\theta = 0.10$, $\beta = 0.30$, and $\alpha = 0.05$. The assumed true-positive rate (30%) corresponds to finding a 50% increase in survival (or disease-free survival, etc) if one has a clinical trial comparing two treatments when there are 25 patients in each group. A review of the published literature during 1977–1979 shows that among published cancer trials, 25 patients per treatment is the median number of patients in a trial. Thus, a "typical" trial has a low sensitivity to finding a 50% increase in survival.

We can change the value of these parameters to determine how these affect the ratio of false-positives to true-negatives. Table 1 summarizes the ratio for a range of different parameter values. The ratios reported in this table are disappointingly high. The lowest ratio, R = 0.20 (which corresponds to

TABLE 1.—Summary of ratio of false-positive to true-positive treatments over a range of parameters (false-positive rate is 5%)

	Ratio when θ† is —		
β*	0.05	0.10	0.20
0.3	3.2	1.5	0.67
0.5	1.9	0.9	0.40
0.9	1.0	0.5	0.25
1.0	0.95	0.45	0.20

*β = probability of detecting a true-positive treatment.

†θ = a priori probability that clinical trial will result in a significant advance.

the probability of 17% for a positive result being, in truth, false), is achieved with values of $\theta = 0.20$ and $\beta = 1.0$. A value of $\beta = 1.0$ reflects an experiment with a very large number of patients. (Conceptually, to achieve a value of $\beta = 1.0$, an infinite number of patients would be required.) The 20% prior probability of success is a relatively high expectation of success. If, indeed, the prior probability of success was that high, then a large number of new therapies with substantial patient benefit would be adopted. This does not seem to be the case with cancer.

The problem of reducing the value of R is limited by the adoption of the 5% false-positive rate. If one reduced the false-positive rate, then the true-positive rate (power) of the trial would be lowered. If one increased the number of patients, the true-positive rate would be raised. Table 2 summarizes the ratio of false-positives to true-positives over a range of different parameters when the false-positive rate is lowered to $\alpha = 0.01$. Note that with a value of $\theta = 0.10$ and sample sizes > 300, the ratio of false-positives to true-positives is approximately 10%, ie, for every ten true-positive treatments in the clinic, there will be one false-positive treatment. A ratio of $R = 0.1$ seems to be a desirable goal for using treatments in the clinic. Note that even with a prior expectation of success of $\theta = 0.20$, a clinical trial will require > 100 patients to achieve this goal.

Finally, what can we conclude from our study of the strategy of clinical trials? The obvious conclusions are as follows.

(a) *Do not initiate a definitive clinical trial unless there is a reasonable a priori probability > 0.05 that a clinically important gain may exist.* One way of interpreting this rule is to carry out pilot studies before launching a definitive study. If the pilot studies are encouraging, then proceed with a large comparative study.

(b) *Comparative trials should be planned with a minimum of 100–200 patients per treatment.* Trials with fewer patients are likely to produce more false-positive results than true-positive results.

To these two recommendations, we can add a third:

(c) *all positive results should be independently confirmed.* This will lower the false-positive rate and raise the true-positive rate. Physicians in practice should exercise caution in adopting a new therapy if there is no independent confirmation.

TABLE 2.—Summary of ratio of false-positive to true-positive treatments over a range of parameters (false-positive rate is 1%)

Sample size*	Ratio when θ† is —		
	0.05	0.10	0.20
100	0.66	0.31	0.14
200	0.31	0.15	0.06
300	0.23	0.11	0.05
400	0.20	0.10	0.04
500	0.20	0.09	0.04

*Refers to total sample size of trial. Each treatment group contains one half of total No. of patients.

†θ = a priori probability that clinical trial will result in a significant advance.

ALTERNATIVES TO CLASSICAL RANDOMIZED TRIALS

Randomized clinical trials are regarded as the most credible way of generating scientific data to compare the benefits of different therapies. However, randomized studies present difficulties in their execution. Often physicians are unwilling to participate in such studies because they do not wish to inform the patient that the treatment will be chosen by a chance mechanism. They feel that such a discussion may compromise the physician-patient relationship. This section discusses alternatives to classical randomized trials that do not change this relationship.

The alternative experimental designs are based on the paper by Zelen (1); they have been termed "randomized consent designs" or "prerandomized designs." Problems of stratification will not be discussed as this will detract from the main conceptual issues.

These designs are put forth in situations where patient consent must be obtained whenever the treatment deviates from normal practice. In the US, the regulations are very specific about requiring consent whenever the patient would be at increased risk because of deviations from normal or standard practice.

The discussion will be simplified to include only a comparison of two treatments that will be designated A and B. Figure 1 shows the experimental design for a conventional randomized study. Figure 2 shows the modification if patient consent is required. Even though physician and patient selection biases are present, the randomization equally distributes these biases (on the average).

Suppose that treatment A is the best standard treatment and B represents an experimental treatment. Treatment A is what a patient would expect to receive under normal circumstances. Figure 3 depicts the randomized consent design for this kind of trial. It is called a "single consent randomized design."

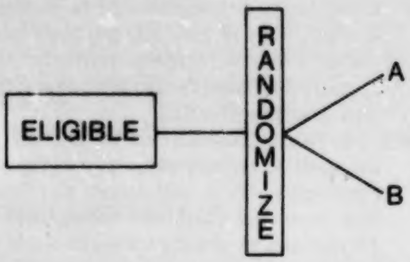

FIGURE 1.—Classical randomized design without patient consent.

After the patient's eligibility is established, the patient is randomized into one of two groups. Patients randomized for the "do not seek consent" group are not approached for consent to enter the clinical trial; they receive the best standard therapy (A). Patients assigned to the "seek consent" group are asked if they wish to participate in the clinical trial and are willing to receive the experimental therapy B. All potential risks, benefits, and treatment options are explained. If the patient agrees, the experimental treatment (B) will be given; if the patient declines to receive the experimental treatment, the patient will (presumably) receive the best standard treatment (A).

The proposed new design has the desirable feature that the physician need approach the patient only to discuss a single therapy. The physician need not appear, in the eyes of the patient, not to know what he is doing and to be "tossing a coin" to decide the treatment. Thus, the patient-physician relationship is not compromised. On the patient's side, there is also an important advantage: before providing consent the patient knows which treatment will be given. Many patients agree to participate in a randomized study but have reservations about continuing after the treatment is known to them. At this point, some patients decline treatment and are considered "cancelled patients." However, others may continue the treatment, despite their reservations, because of the built-up momentum to do so and their reluctance to renege on their consent. This design requires a decision by the patient only on the experimental treatment. Hence, the patient's decision-making processes should be more straightforward. This new design cannot be used when there are important reasons for conducting a "double-blind" experiment, ie, a trial in which neither the physician nor the patient knows the identity of the treatment during the course of treatment or its evaluation.

The analysis of this new design requires that group 1 (receiving only treatment A) be compared with group 2 (receiving treatment A or B). In other words, the comparison must be made with all patients in group 2, regardless of which treatment each received. Including all patients dilutes the measurable effect of treatment B. Nevertheless, all patients must be included if the analysis is to provide a valid comparison with treatment A. If only a small proportion of patients are willing to take treatment B, this experimental plan may be useless in evaluation of this treatment. However, the refusal of a large proportion of patients to agree to accept treatment B may be interpreted to indicate that it was premature to introduce the experimental therapy into a clinical trial.

Figure 4 depicts another kind of randomized consent design. We call this a "double consent randomized design." It is suitable for comparing two treatments in which there is no control or best standard treatment. Patients are randomized to each of the two treatments and then are asked if they wish to accept the randomized treatment. If they decline, they are given the alternate treatment or perhaps another treatment not under investigation in this

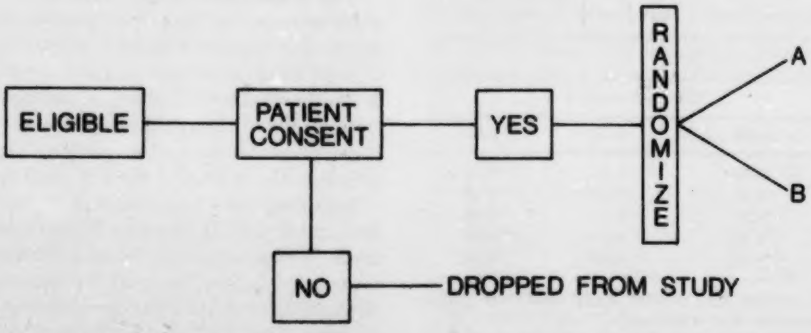

FIGURE 2.—Classical randomized design with patient consent.

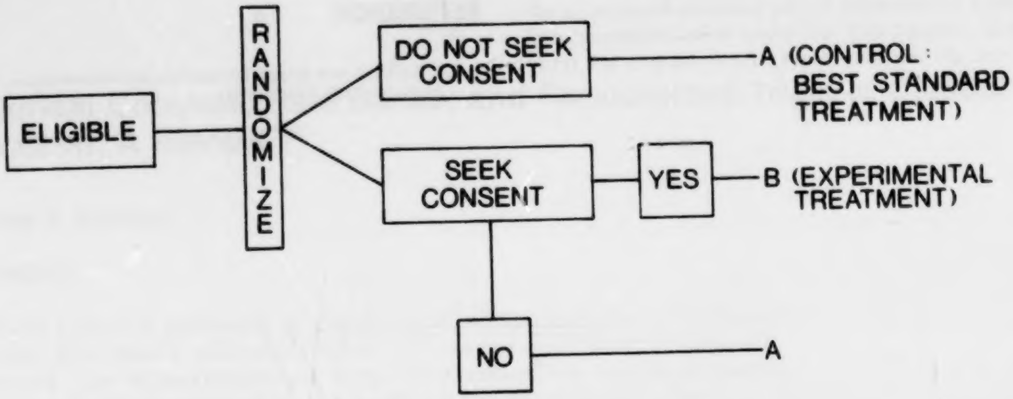
ELIGIBLE
RANDOMIZE
DO NOT SEEK CONSENT
A (CONTROL: BEST STANDARD TREATMENT)
SEEK CONSENT
YES
B (EXPERIMENTAL TREATMENT)
NO
A

FIGURE 3.—Single randomized consent design.

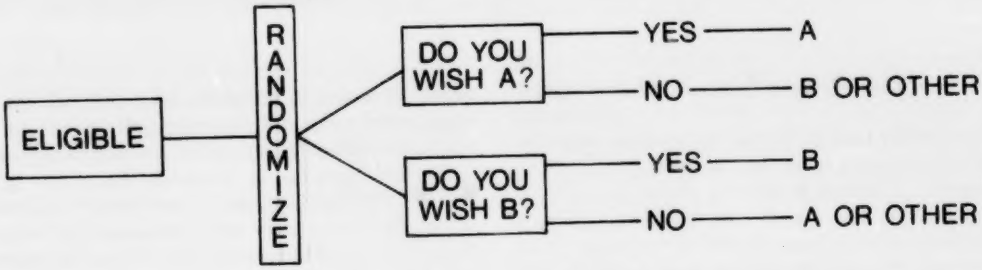
ELIGIBLE
RANDOMIZE
DO YOU WISH A?
YES — A
NO — B OR OTHER
DO YOU WISH B?
YES — B
NO — A OR OTHER

FIGURE 4.—Double randomized consent design.

study. Comparison of the two treatments is made by comparing groups 1 versus 2 regardless of the treatment actually received.

Table 3 summarizes the efficiencies of the single and double consent randomized design. These efficiencies depend on the probability of acceptance of the designated treatment by the patient. For example, consider a single consent randomized design in which only 50% of the patients accept the experimental treatment. The efficiency of the design is 25%. This means that four times as many patients are required to obtain the same sensitivity in this design as in a conventional randomized design. Thus, unless accrual is increased by at least a factor of four, this design may not be useful. This factor of four is called the "break-even accrual factor" in table 3.

Such experimental designs for carrying out clinical trials are beginning to receive attention in the US. I believe that these designs will dominate the field of randomized controlled clinical trials. They preserve the physician-patient relationship and also

TABLE 3.—Efficiencies of single and double consent randomized designs

Probability of acceptance	Single consent		Double consent	
	Efficiency	Break-even accrual factor	Efficiency	Break-even accrual factor
0.50	25%	4	0	—
0.60	36%	2.8	4%	25
0.70	49%	2.0	16%	6.2
0.80	64%	1.6	36%	2.8
0.90	81%	1.2	64%	1.6
0.95	90%	1.1	81%	1.2

are more informative to the patients because in advance of consent they will know what treatment will be given. At the same time, these designs are truly randomized.

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Historical Controls, Data Banks, and Randomized Trials in Clinical Research: A Review[1,2]

Thomas R. Fleming[3,4,*]

SUMMARY

A brief review is presented of the strengths and weaknesses of historical controls, data banks, and randomized trials in the evaluation of clinical treatment. Use of prerandomized versus postrandomized informed consent is discussed. Recommendations are made for the development of an appropriate clinical research strategy.

[Cancer Treat Rep 66:1101–1105, 1982]

The use and the sophistication of scientific techniques in clinical experimentation have increased. In the words of Marvin Zelen (1), "The day is rapidly receding when ex-cathedra judgments dominate therapeutics." However, a continual problem faces the clinical investigator who wants to evaluate a new treatment and to compare its efficacy with that of an existing "standard" treatment. How can information enabling this comparison be obtained in a manner that is ethically acceptable, that allows one to make an unbiased or fair evaluation, and that is the most efficient possible? Here, "efficiency" refers to minimizing the number of patients and medical personnel, the amount of money, and the length of time needed for the assessment. Methods for providing the necessary information include the use of a contemporary treatment group compared with historical controls, the use of data banks in which patients with and without the treatment in question are compared, or the performance of randomized trials. In the last-named instance, informed consent either is always obtained before the time of randomization or is only obtained after. In this volume, these four methods are discussed by Gehan (2), Starmer and Lee (3), Simon (4), and Zelen (5), respectively.

These and many other investigators have explored the strengths and weaknesses of each methodologic approach in clinical research. These issues are considered in the second and third sections of this paper. In the fourth section, the general problem of developing an appropriate clinical research strategy is addressed and recommendations are made.

Strengths and Weaknesses of Methods to Select Controls—A Brief Review

In an attempt to make efficient use of limited research resources, certain clinical trials are designed to use historical controls. This design is particularly appropriate when one evaluates experimental treatment regimens that are not only hoped to be but also expected to be clearly superior to standard therapies.

Unfortunately, substantial heterogeneity often exists in the patient population eligible for any given clinical trial, particularly in cancer research. Thus, whenever historical controls are used, great care must be taken to minimize selection bias, that is, to minimize the lack of comparability between the experimental treatment group and the historical control group. To this end, Pocock (6) has suggested important criteria to be implemented in designing a historically controlled study. Gehan and Freireich (7) have emphasized the role of quantitative models that can be used during the comparison of treatment groups to adjust for the intergroup dissimilarities

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[1] Received Mar 4, 1982; accepted Mar 4, 1982.

[2] Supported by Public Health Service grant CA-24089 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Medical Statistics and Epidemiology, Mayo Clinic and Mayo Foundation, Rochester, MN.

[4] I express my appreciation to the many colleagues at Mayo who provided helpful comments on this paper.

[*] *Reprint requests to*: Thomas R. Fleming, PhD, Department of Medical Statistics and Epidemiology, Mayo Clinic, Rochester, MN 55905.

in identified patient characteristics influencing prognosis. Unfortunately, as noted by Simon (4), Byar (8), Pocock (9), and Peto (10), much of the heterogeneity in the study population is not accounted for by the prognostic variables that are known and properly recorded. Thus, when using historical controls, one must exercise great caution in drawing conclusions about treatment comparisons. One cannot distinguish differences truly due to treatment from differences due to systematically occurring intergroup dissimilarities arising from important but unrecognized or unrecorded covariates.

Random assignment of patients to treatment groups eliminates the systematic occurrence of lack of comparability among these groups. This property is referred to as "unbiasedness" by Simon (4). Randomly occurring dissimilarities in recorded or unrecorded covariates can still appear among treatment groups in randomized trials, although in very large samples these dissimilarities also disappear. Simon calls this property "consistency." The property of unbiasedness, particularly in the elimination of systematic dissimilarities that occur among treatment groups and that are caused by unknown or unrecorded prognostic factors, is a fundamental component to a fair, reliable, and reproducible evaluation of treatment. Only randomization guarantees unbiasedness.

Certainly, an increased investment of research resources is required to perform a randomized trial. However, this increased investment, together with the unbiasedness and increased reliability of results obtained from such a design, leads to a greater willingness to publish not only the positive results but also the negative results. This lessens the reporting bias that arises in clinical research when, as Zelen (5) states, "Editors and even authors are reluctant to publish negative results."

With the rapid and extensive development of computer hardware and software as well as statistical methodology, the use of data banks has been supported in some treatment evaluations as an alternative to randomized or historically controlled trials. The data bank or data-based approach, when constructed in the careful manner followed by Starmer and Lee (3), is indeed useful. The data base, a shared repository of data elements describing attributes of patients, interventions, and outcomes and collected uniformly over long periods, is often well-suited for identifying prognostically important variables, for studying temporal shifts in patients' disease and in technology, and for investigating the feasibility and desirability of performing confirmatory randomized trials of certain comparisons of treatment. Data bases that are population-based can be used as well in the assessment of incidence and mortality of cancers.

In chronic disease, Starmer and Lee (3) propose that the data-based approach be used for treatment evaluation and individualized selection of treatment because the prolonged course between onset, intervention, and target outcome and the nonstationary disease process and milieu render randomized designs impractical. However, the concerns of Simon (4) and Byar (8) about bias in treatment assignment, missing data, and lack of standardization of treatment and response assessment should be carefully heeded when data-based treatment evaluation is considered. Bias will arise whenever treatment decisions are made not only on the basis of elements in the data bank but also on other important but unrecorded considerations. In addition, how data-based, individualized selection of treatment could result in the selection of newly proposed therapies is uncertain.

Prerandomized or Postrandomized Informed Consent?

Are the treatments potentially medically equivalent? This is a key ethical question that must be addressed when one is considering whether to randomize patients in a cancer clinical trial. If the answer is yes and the decision is made to conduct such a trial, the Code of Federal Regulations (45 CFR 46) requires, in essence, that each patient who could be asked to receive therapy "which departs from . . . established or accepted methods" give informed consent, the purpose of which is to facilitate and guarantee informed decisions from each participating patient. Information to be provided to the patient includes a discussion of the risks and benefits of each of the treatments in the study and of potentially effective alternative therapies.

The process of obtaining informed consent is often lengthy. This, together with Zelen's observation (1) that "physicians may find it difficult to tell patients that they do not know which treatment is best," has resulted in substantially reduced accrual rates in many randomized trials.

To alleviate this problem, Zelen (1) has proposed that patients be randomized before informed consent is obtained. Supposing randomization is to either a "standard" or an "experimental" treatment, he suggests that consent be obtained only from those randomized to the experimental regimen. Patients randomized to the experimental regimen must be analyzed with that group whether or not they consent to that treatment. Even though this dilutes true differences in treatment, Zelen's design could yield an increase in efficiency over the classic prerandomized informed consent design if it leads to an increased rate of accrual and if the consent rate is fairly high among those randomized to the experimental group.

Zelen's design (1) presents some ethical problems not present in the classic situation wherein randomization occurs only after informed consent has been obtained. The first problem concerns those patients who are randomized to the experimental group. Recall that, ethically, randomization requires that investigators have decided that the treatments being compared are potentially medically equivalent. In turn, this assessment of equivalence should be conveyed to the patients if they are to be fully informed. However, to increase the efficiency of the study, investigators need as many patients as possible who are randomized to the experimental regimen to elect to receive that treatment. Thus, the design provides a subtle encouragement for the investigator to provide a biased presentation of the relative merits of the treatments to these patients.

The second ethical problem concerns those patients who are randomized to the standard regimen. In Zelen's single randomized consent design (*see* fig 2 in Zelen [5]), these patients are not approached for consent to enter the clinical trial. He states, "The physician need only approach the patient to discuss a single therapy. The physician need not leave himself open, in the eyes of the patient, to not knowing what he is doing. . . ." (1). Important ethical questions must be addressed. Do these patients, as well, have a need for informed consent? Recall that the intent of the informed consent process is to provide the patient with a discussion of the risks and benefits of the various therapeutic options in order to facilitate and guarantee informed decisions. Doesn't this need exist, then, whenever there is more than one potentially effective therapy for a given patient, in fact, even for the individual being considered for treatment in a nonresearch setting? Fost (11) has stated, "To initiate any treatment, standard or experimental, without the patient's fully informed consent is to fail to respect him as a person with a right to autonomy." If one accepts this statement, it follows that patients who are randomized to the standard regimen in Zelen's design (1) continue to have the same need and right to be fully informed and to formally consent. If this right is satisfied, the newly proposed design usually will lose much of its appeal. An example of this issue is early breast cancer in which the standard regimen in a given trial is modified radical mastectomy and the experimental regimen is excision plus radiation therapy. Certainly, under any circumstances, a woman being considered for mastectomy has a right to be informed of potentially effective alternative therapies.

In summary, for Zelen's design (1) to be useful, one must identify situations in which patients receiving a certain treatment are ethically required to be fully informed of alternative therapies and situations in which another treatment exists that is potentially medically equivalent to the first but for which it is acceptable not to inform patients of the alternatives. In addition, one must anticipate that a high proportion of patients who are randomized to the treatment requiring consent will elect to receive it.

Appropriate Strategy in Clinical Research

In the words of Simon (4), "Good clinical therapeutic research identifies useful treatments, provides reliable leads, or appropriately categorizes useless or harmful treatments." If one accepts this statement as the general goal in clinical research, one must consider what Simon refers to as the "thermodynamics of clinical trials," that is, the aggregate results from a program of clinical research. Simon (4) and Zelen (5) have provided clear illustrations of the importance of this consideration.

Well recognized in the design of a clinical trial is the need to properly control the size (α) and the power ($1-\beta$) of the trial. Here α denotes the false-positive probability, that is, the probability of concluding that differences exist when, in fact, there are no true differences, and $1-\beta$ denotes the probability of detecting clinically meaningful differences that truly exist. However, not as commonly considered when the trial is designed is π, the pretrial estimate of the probability that clinically important true differences exist. Knowledge of α, $1-\beta$, and π permits the evaluation of such important questions as "What is the probability that a positive result in our trial will be a true positive result?" One can see in table 1 that the answer is

$$\frac{\pi(1-\beta)}{\pi(1-\beta)+(1-\pi)\alpha}.$$

TABLE 1.—Relationship of experiment results and truth

Result of experiment	Truth		
	Positive	Negative	
Positive	$\pi(1-\beta)$	$(1-\pi)\alpha$	$\pi(1-\beta)+(1-\pi)\alpha$
Negative	$\pi\beta$	$(1-\pi)(1-\alpha)$	$\pi\beta+(1-\pi)(1-\alpha)$
	π	$1-\pi$	1

In many clinical situations (*see* Simon [4] and Zelen [5]), a realistic pretrial estimate of the probability that true clinically meaningful differences exist is $\pi = 0.20$ (*or 0.10*). Then, even if trials are constructed to have a size of only $\alpha = 0.05$ and a power as high as $1 - \beta = 0.80$, 20% (*or 36%*) of positive results will be false-positive. Unfortunately, the actual situation is often even worse. Studies may be designed to have sample sizes adequate to provide a size of $\alpha = 0.05$ and a power of $1 - \beta = 0.80$ if the patient population is homogeneous. Typically, however, the sample of patients is quite heterogeneous, causing a loss in power. Additional false-positive and false-negative conclusions are introduced when trials are terminated early as a consequence of interim analyses based on test procedures that are appropriate only if applied at the predetermined time of final analysis. The impact of this is that the clinical trial, as actually carried out, may have a size closer to $\alpha = 0.20$ and a power closer to $1 - \beta = 0.60$, which, if $\pi = 0.10$, would result in 75% of positive trials being false-positive.

One other very common source of false-positive conclusions is the consideration of data-suggested hypotheses arising from "data dredging" or "exploratory data analysis." As mentioned earlier, the population of patients receiving treatment is generally quite heterogeneous. Since it is well recognized that the relative benefits of various treatment regimens may vary from one type of patient to another, it is common practice to perform a retrospective search through the data to find subsets of patients showing large differences in treatment. This retrospective search, referred to as exploratory data analysis, can be instructive, yet all too frequently it provides false leads, as illustrated by Starmer and Lee (3).

What, then, does constitute an effective strategy in clinical research? The key, discussed by Gehan (2), Starmer and Lee (3), Simon (4), and Zelen (5), is the recognition of the need for independent confirmatory trials. Simon speaks of treatment evaluation and comparisons occurring in two stages. Stage I comprises small- or moderate-sized screening trials that would encourage innovation. In some situations, these trials should be randomized. Yet it is in the setting of screening trials that historically controlled or data-based trials are useful as well. The increase in efficiency of carefully performed historical and data-based designs could outweigh their lack of "unbiasedness," since a confirmatory trial (or trials) would be performed to verify results.

Unless results in stage I provide extreme evidence in favor of differences in treatment, all positive trials should then be confirmed in stage II in large, randomized clinical trials. These trials generally should have at least 100–200 patients in each treatment group. Performing these large, definitive stage II confirmatory trials only after positive stage I studies ensures that Zelen's conclusion (5) (*a*) will be met, that is, definitive clinical trials should be initiated only if π, the pretrial estimate of the probability that clinically important gains do exist, is > 0.05.

Since only positive stage I trials will be confirmed in stage II, the power, $1 - \beta$, of the stage I studies should be high enough that frequent missing of clinically important true differences is avoided. Specifically, in the screening trial, $1 - \beta$ preferably should be at least 0.90. As a result, since sample sizes in screening trials are small or moderate, the size of a stage I screening trial often needs to be > 0.05.

Ethically required interim analyses should be performed during these large stage II trials by methods that maintain the desired upper limit on the number of false-positives and that maintain sensitivity to treatment-caused differences of interest (*see* Fleming et al [12]).

It is in the setting of the large stage II trials that sufficient data exist for one to perform useful exploratory data analyses. Since as much as possible needs to be learned from the data, this practice of retrospective exploration of the data should be continued for as long as investigators recognize that the only hypotheses being definitively evaluated by the stage II trial are those pretrial hypotheses that motivated the study. Data-suggested hypotheses formulated during exploratory analyses of data from stage II trials should in turn be confirmed in subsequent trials.

One of the greatest causes of difficulty in carrying out this two-stage clinical research strategy is the tendency to overreact to positive results obtained in the small- or moderate-sized stage I screening trials. In manuscripts, these results often are presented as though they provide a reliable assessment of treatment rather than screening information requiring confirmation. In fact, this overreaction occasionally has led to changes in medical practice so firmly accepted that it has been judged to be unethical to consider randomization against future alternative treatments. It is useful when obtaining these positive screening results to recall the very high chance that a positive result in a trial of this size will actually be a false lead and the subsequent negative impact of accepting an inferior treatment. In the words of Artemus Ward, "It isn't so much the things we don't know that get us in trouble. It's the things we know that aren't so."

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Prognostic Significance of the Lukes and Collins Classification in Patients Treated With COMLA[1,2]

Donald L. Sweet,[3,*] Robert D. Collins,[4] Richard S. Stein,[4] and John E. Ultmann[3]

SUMMARY

The Lukes and Collins classification was applied to 47 patients who received COMLA (cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine) as initial treatment for diffuse histiocytic lymphoma (DHL). Pathologic staging was complete in 39 of 47 patients; two patients had stage II_E disease, 17 had stage III, and 28 had stage IV. Complete remission, which was documented by extensive clinical restaging, was achieved in 25 (64%) of 39 patients with morphologic B-cell lymphomas and in only three of eight patients with non-B-cell lymphomas. The median duration of complete remission is significantly longer in patients with B-cell lymphomas than in those with non-B-cell lymphomas (45+ versus 13 months, $P < 0.01$). Among patients with DHL, median survival is significantly longer for those with B-cell lymphomas than for those with lymphoma of T-cell or indeterminate origin (60+ versus 6 months, $P < 0.01$). While DHL is regarded as a curable disease, patients not achieving complete remission are rarely salvaged; median survival for these patients was 10 months. The Lukes and Collins classification allows us to identify patients with DHL in whom newer therapies are needed for initial treatment. Further study may enable us to identify additional unfavorable subsets in the B-cell category.

[Cancer Treat Rep 66:1107–1111, 1982]

In 1974, Lukes and Collins suggested that the immunologic origin of the malignant lymphomas can be recognized morphologically and can constitute the principal criterion of a classification for the lymphomas (1). They also suggested that, but for rare exceptions, the histiocytic lymphomas typically demonstrate the features of transformed lymphocytes. Although the clinical-pathologic utility of the Rappaport system has been demonstrated, there are limited data regarding the clinical utility and predictive value of the Lukes and Collins classification. In this report, we describe the results of a retrospective analysis of 47 patients who received identical chemotherapy for the treatment of advanced diffuse histiocytic lymphoma (DHL). These patients have had their histology reclassified by one of us (R. D. Collins) by the criteria of Lukes and Collins. The response to therapy and survival for these patients is the subject of this report.

PATIENTS AND METHODS

Between March 1974 and March 1979, 55 patients at the University of Chicago Hospitals and Clinics

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[1] Received Oct 8, 1980; revised Nov 2, 1981; accepted Nov 18, 1981.

[2] Supported in part by Public Health Service grants CA-14599, CA-16401, and CA-19429 (National Cancer Institute) and AM-07134 (National Institute of Arthritis, Metabolism, and Digestive Diseases) from the National Institutes of Health, Department of Health and Human Services; the Beverly Duchossois Fund; the Goldblatt Brothers Employees Nathan Goldblatt Cancer Research Fund; and the Jean Heiman Fund. Dr. Sweet is a junior faculty clinical fellow of the American Cancer Society.

[3] Department of Medicine and Cancer Research Center, University of Chicago, IL.

[4] Departments of Pathology (*R. D. Collins*) and Medicine (*R. S. Stein*), Vanderbilt University School of Medicine, Nashville, TN.

**Reprint requests to:* John E. Ultmann, MD, The University of Chicago Cancer Research Center, Box 444, 950 E 59th St, Chicago, IL 60637.

and at the Vanderbilt University Hospital were treated for advanced DHL with cyclophosphamide, vincristine, methotrexate, leucovorin, and cytarabine (COMLA). At the University of Chicago Hospitals and Clinics, 42 consecutive unselected patients with DHL received COMLA (2). Of these, 34 had pathologic material available for review. Patients at Vanderbilt University considered to have histiocytic lymphoma had been entered in a randomized trial between COMLA and the combination of bleomycin, doxorubicin, cyclophosphamide, vincristine, and prednisone from June 1977 to September 1979. Of these patients, 13 received COMLA and had material available for histologic review.

All hematopathologic material was reviewed by one of us (R. D. Collins) and classified according to the morphologic criteria of Lukes and Collins. As in other publications by our group, we have substituted the term large transformed cell lymphoma for the term large non-cleaved cell lymphoma (3,4). In patients whose lymphomas had both large cleaved and large transformed cells, we used previously reported criteria and categorized the cases as transformed cell lymphomas if > 25% of the cells were transformed lymphocytes (3).

Large transformed cell lymphomas may be considered to be of follicular center cell origin when cleaved cells are present or when there is residual follicular nodulation. Patients whose lymphomas were composed of large transformed lymphocytes but did not meet these criteria or the criteria for node-based, T-cell lymphoma were categorized as having "large transformed cell lymphoma of indeterminate origin" (5).

While morphologic criteria were used for classification, studies of surface immunoglobulin and E-rosettes were performed in six patients treated at the Vanderbilt University Hospital (6). It was confirmed that three patients diagnosed by morphologic criteria as having follicular center cell lymphomas had monoclonal B-cell lymphomas and that three patients diagnosed as having immunoblastic sarcoma of T cells had T-cell lymphomas. Conceivably, immune marker studies might have been of value in classifying the patients categorized as having large transformed cell lymphoma of indeterminate origin, but marker studies were not performed in these patients.

The pathologist reviewing the cases was aware only that the patients had been originally classified as having DHL according to the criteria of Rappaport but was unaware of stage or survival. Seven patients from the Chicago study were inevaluable; one was reclassified as having malignant histiocytosis, and the other six had insufficient histologic material for review. Patients from Vanderbilt University were included if they had histologic diagnosis that would have been labeled as histiocytic lymphoma by Rappaport.

These criteria describe tumor cells that are large lymphoid cells (20–40 μ) with vesicular nuclei, inconspicuous or absent nucleoli, and variability in size. Fewer than 25% of the cells were small cells. By definition, cells of undifferentiated type, lymphoblastic lymphoma, Burkitt's lymphoma, and malignant histiocytosis are not included in these criteria. Patients with nodular histiocytic or mixed cell lymphoma or patients with two histologies were not included in this study. Staging criteria were those suggested by the Ann Arbor conference on Hodgkin's disease. Pathologic staging was complete in 39 of the 47 patients. Two patients were classified as having stage $\mathrm{II_E}$ disease; 17, stage III; and 28, stage IV.

The COMLA regimen consists of high-dose cyclophosphamide (1.5 g/m^2) administered on Day 1 and vincristine (1.4 mg/m^2) on Days 1, 8, and 15; methotrexate (120 mg/m^2) and cytarabine (300 mg/m^2) for 8 consecutive weeks, on Days 22, 29, 36, 43, 50, 57, 64, and 71; and leucovorin rescue (25 mg/m^2 orally) beginning 24 hours after the methotrexate infusion, for four doses, 6 hours apart. A 2-week rest period was allowed and the cycle was repeated. Three cycles were administered. All of the agents except leucovorin were administered as single iv injections over 5–10 minutes. The order of methotrexate and cytarabine administration was random. Renal function was monitored every 2–3 months. During the first rest period, the major parameters of the disease were assessed. One month after completion of the third COMLA cycle, all patients believed to be in complete remission clinically were re-evaluated for the presence of disease. This restaging procedure included the re-examination of previously abnormal areas detected by physical examination, X-ray studies, scintiscans of liver and bones, or biopsies of the bone and/or liver. Residual or suspicious lymphadenopathy or other disease was biopsied. Complete remission was defined as the disappearance of all disease. No maintenance therapy was given. Partial remission was defined as a $\geqslant$ 50% reduction in the measurable tumor lasting $\geqslant$ 2 months. Patients achieving partial remission continued to receive COMLA until there was objective evidence of disease progression.

Survival was calculated from the date of diagnosis to the most recent followup as of August 1981. The remission duration was determined from the date of the first objective complete remission to documented relapse. The data were analyzed by the life-table method, and life curves were compared using the generalized Wilcoxon test as modified by Gehan

(7). Subgroup evaluation was made using the Fisher exact test.

RESULTS

Table 1 shows the results of reclassification according to the criteria of Lukes and Collins and the corresponding response to therapy of these 47 patients. Thirty-nine patients were believed to have B-cell lymphoma (follicular center cell origin in 35 and immunoblastic sarcoma of B-cell type in four). Eight patients had non-B-cell lymphomas, including three with immunoblastic sarcoma of T cells (confirmed by sheep E-rosettes) and five with large transformed cell lymphoma of indeterminate origin.

There was no relation of cell origin to the presence or absence of constitutional symptoms, sex, or age, and the two groups of patients did not differ significantly with respect to the incidence of previously identified unfavorable prognostic features such as bone marrow involvement or massive abdominal disease (table 2).

There was no significant difference in the likelihood of achieving complete remission in patients with different stages of disease. The rate of achieving complete remission was 73% for patients in stage II_E or III and 50% for patients in stage IV ($P = 0.15$). While patients with B-cell lymphoma were younger, this is unlikely to have affected results since the complete remission rate was 54% in patients $\leqslant 60$ years old and 69% in patients > 60 years ($P = 0.52$).

The complete remission rate for the patients with B-cell lymphomas was 64.1% (25 of 39) and the median survival was 60+ months. Only three of eight patients with lymphoma of non-B-cell origin achieved complete remission, with an observed median survival of 6 months. Nineteen of 39 patients with B-cell tumors remain in complete remission compared to only one of eight with non-B-cell lymphomas. The median duration of complete remission was significantly longer for patients with B-cell lymphomas than for those with non-B-cell lymphomas (45+ versus 13 months, $P = 0.03$). The median survival was significantly longer in patients with B-cell lymphomas than in patients with lymphomas of T-cell or indeterminate origin (60+ versus 6 months; $P < 0.01$, two-tailed test). Survival was similar for patients with large cleaved cell lymphomas and large transformed cell lymphomas.

DISCUSSION

The outlook for patients with DHL has improved considerably over the past decade. However, despite the availability of excellent polychemotherapy regimens producing high rates of complete remission and perhaps 50% cure rates, a large number of patients appear to be resistant to therapy. DHL is a heterogeneous group of diseases, and a number of factors related to histologic identification may be important in helping to identify those patients with histiocytic lymphoma who are not responsive to current chemotherapeutic regimens. For example, Miller et al (8) have shown that patients with DHL and sclerosis are a distinct histologic subtype with a poor prognosis.

In this study, we have attempted to determine whether or not the immunologically oriented classification of Lukes and Collins could be used to identify patients with histologic subgroups that demonstrate a survival advantage or distinct pattern of clinical behavior. The data presented here demonstrate that patients with non-B-cell characteristics,

TABLE 1.—Complete response rate and survival by histologic category

Histology	No. of patients	Complete response (%)	Median survival (mos)
B-cell lymphomas			
Follicular center cell lymphomas			
Large cleaved cell lymphoma	9	5 (56)	60+
Large transformed cell lymphoma	26	17 (65)	60
Immunoblastic sarcoma of B cells	4	3 (75)	24
Total	39	25 (64)	60
Non-B-cell lymphomas			
Immunoblastic sarcoma of T cells	3	1 (33)	15
Large transformed cell lymphoma of indeterminate origin	5	2 (40)	6
Total	8	3 (38)	6

TABLE 2.—Characteristics of patient groups*

Characteristic	B-cell lymphoma	Non-B-cell lymphoma	P value
Stage			
$\mathrm{II_E}$, III	17	2	0.45
IV	22	6	
Male	17	5	0.44
Female	22	3	
Symptoms	25	5	1.00
No symptoms	14	3	
Median age (yrs)	47	59.5	0.004
Bone marrow involvement	2	2	0.12
Massive abdominal disease	1	0	—

*Unless otherwise specified, values = No. of patients.

either immunoblastic sarcoma of T-cell origin or large transformed cell lymphoma of indeterminate origin, have a poor prognosis. We acknowledge that the size of the non-B-cell group is small. Patients with follicular center cell lymphomas have an excellent prognosis when treated with the COMLA regimen and can expect disease-free survival > 5 years if they achieve complete remission.

Recently, Bloomfield et al (9), combining the Lukes and Collins classification with cell surface marker analysis, have demonstrated a significant survival advantage for those patients whose lymphoma is classified by surface markers as B-cell type compared to those with T-cell or null cell type. This analysis, however, was not limited to histiocytic lymphoma. Strauchen et al (10) have suggested that subclassifying patients with DHL according to cell origin may have clinical utility. Their series, however, included patients with histologies other than DHL, and various forms of therapy were employed. Their data did suggest, however, that there was a distinct survival advantage for patients with large cleaved cell lymphomas and an intermediate prognosis for those with large non-cleaved cell and mixed follicular center cell tumors.

Armitage et al (11) have also published an analysis of the subclassification of histiocytic lymphoma. In that series, however, large cleaved cell lymphoma appeared to have a relatively unfavorable prognosis. Since many patients with large cleaved cell lymphoma might be classified as having mixed cell lymphoma under the Rappaport system, selective inclusion or exclusion of patients might explain the divergent prognosis of large cleaved cell lymphomas noted in this series. Alternatively, specific subtypes of DHL may respond differently to different chemotherapy regimens.

Warnke et al (12) have suggested that patients with large cell lymphoma in which the tumor cells are negative for surface immunoglobulin have a longer survival than patients whose cells are immunoglobulin-positive. The clinical relevance of this report is uncertain, since the study is retrospective and the patients were selected. The results of this investigation underline the immunologic heterogeneity of the large cell lymphomas, as our report demonstrates.

The results of these studies, as well as the data presented here, suggest that the prognosis for patients with lymphomas of non-follicular center cell origin is significantly less favorable than for those with lymphomas of follicular center cell origin. These differences do not appear to be accounted for by extranodal sites of disease, stage, or the presence or absence of constitutional symptoms.

Patients with DHL treated with COMLA demonstrate, upon reclassification by the criteria of Lukes and Collins, a distinct survival advantage if they have histologies consistent with follicular center cell or B-cell origin. The patients with non-B-cell lymphomas have a dismal prognosis and should be identified for alternative forms of initial management. The term histiocytic lymphoma as described by Rappaport includes a heterogeneous group of lymphomas that can be subclassified on the basis of morphology. The subsets identified by the Lukes and Collins classification appear to have prognostic significance.

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Limited Squamous Cell Carcinoma of the Lung: A Southwest Oncology Group Randomized Study of Radiation With or Without Doxorubicin Chemotherapy and With or Without Levamisole Immunotherapy[1,2]

Joel E. White,[3] Timothy Chen,[4] Richard Reed,[5] Joaquin Mira,[5] W. J. Stuckey,[6] Thomas Weatherall,[6] Robert O'Bryan,[3] Michael K. Samson,[7] and H. Gunter Seydel[7,*]

SUMMARY

In 1976, the Southwest Oncology Group activated a four-arm randomized study of limited squamous cell carcinoma of the lung. The purpose of this study was to determine if doxorubicin and/or levamisole added to radiation therapy improved the local control rate and survival in patients with limited squamous cell carcinoma of the lung. Of the 107 eligible patients, 15 (14%) had complete responses and 20 (19%) had partial responses. When compared by chemotherapy, immunotherapy, and performance status, no statistically significant difference was found in response rates or in survival. Although not statistically significant, the survival and response rates of patients in the combined levamisole arms were shorter than those of patients in the combined arms not containing levamisole. Patients receiving radiation therapy alone had the best survival. The irradiated field was the site of failure in 68 (88%) of the 77 patients in whom the site of failure was specified. Fifty (65%) of the patients failed in the radiation therapy field only. Chemotherapy and/or immunotherapy as employed in this protocol were of no benefit.

[Cancer Treat Rep 66:1113–1120, 1982]

Most bronchogenic carcinoma is inoperable for cure at the time of diagnosis. The data of Cutler et al (1) from the National Cancer Institute show that in 31% of patients the tumor is *clinically* confined to one hemithorax (limited or regional disease), but it is inoperable or incompletely resectable. In the squamous cell histology, which accounts for about 40% of bronchogenic carcinoma, a somewhat larger fraction of patients have what appears to be limited disease (with no clinically discernible tumor beyond the hemithorax and ipsilateral node-bearing areas), so that all the grossly apparent tumor may be included in a single radiation therapy portal. Patients with squamous cell histology appear to have modest survival prolongation as a result of radiotherapy: median survival time (MST) and percent 1-year survival for such patients who receive supportive care alone were 3.5 months and 13%, respectively, in the studies from the Veterans Lung Study Group (2), while MST for patients receiving radiotherapy ranges from 4.7 to 14 months (average, 5.5) (3), and the fraction of patients surviving 1 year ranges from 11% to 57% (average, 25%–30%), with a 5%–6% 5-

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[1] Received July 21, 1981; revised Dec 3, 1981; accepted Dec 17, 1981.

[2] Supported by Public Health Service grants CA-04915, CA-03389, CA-22433, CA-14028, CA-20319, CA-04920, CA-13238, CA-13612, CA-22411, CA-03096, CA-12644, CA-27057, CA-12213, CA-21116, CA-28862, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Henry Ford Hospital, Detroit, MI.

[4] Biostatistical Office, Southwest Oncology Group, Houston, TX.

[5] University of Texas Health Science Center, San Antonio.

[6] Tulane University School of Medicine, New Orleans, LA.

[7] Wayne State University, Detroit, MI.

[*] *Reprint requests to:* Southwest Oncology Group, Operations Office, c/o Cancer Treatment and Research Center, 4450 Medical Dr, San Antonio, TX 78229.

year survival. Patients with squamous cell lung cancer have a high locoregional failure rate (4,5). As local control improves, however, the distant failure rate increases (5).

The dual problems of locoregional failure following high-dose radiation therapy and distant metastatic failure have been addressed by various attempts to combine radiation therapy with chemotherapy (6–12). Theoretically, the addition of chemotherapy enhances the sensitivity of local tumor in the chest, thus making it more likely that the radiation will sterilize the tumor. Also, in theory, the addition of chemotherapy when systemic metastases are still small microscopic deposits creates a greater likelihood of sterilizing these microdeposits. Most combination studies have been analyzed on the basis of response rates and median survival, usually measured in days or weeks.

Several studies employing combinations of drugs have shown survival prolongation of responders versus nonresponders in patients with extensive disease, both in studies which employed doxorubicin (13) and in those which did not (14). Overall response rates in these studies have ranged from 20% to 42%. None demonstrated an impact on median survival, and none showed clear superiority to doxorubicin alone.

Preliminary studies involving administration of immunoadjuvants with surgery or radiation therapy suggest a survival advantage (15,16).

METHODS AND MATERIALS

Based on the foregoing information, the Southwest Oncology Group activated a four-arm randomized study of limited squamous cell carcinoma of the lung in 1976. The purpose of this study was to determine if doxorubicin and/or levamisole added to radiation therapy improved the local control rate and survival in patients with limited squamous cell carcinoma of the lung.

Patients with no prior chemotherapy or radiotherapy were eligible for entry in the study if they had a histologically proven diagnosis of squamous cell carcinoma of the lung which could not be resected for cure and which was confined, on the basis of all clinical measurements, to the hemithorax and mediastinal and supraclavicular nodes, provided also that such nodes could be included in the same radiotherapy portals as the primary lesion. Also eligible for the study were patients who had had surgical treatment, but in whom gross, measurable, residual tumor remained. Patients who were inoperable for other medical reasons (eg, cardiovascular disease, chronic obstructive pulmonary disease, or senility), but whose cancer was not proven by mediastinoscopy, laminography, or other procedures to be unresectable, were excluded from the study. Patients were required to have measurable lesions, and informed consent was obtained in accordance with institutional practice. Patients were stratified by performance status according to a modified Karnofsky scale and according to whether they were inoperable or had had an incomplete resection. Weight loss and supraclavicular lymph node involvement were recorded but not utilized as a stratification variable. The pretreatment evaluation included a complete history and physical examination, chest X-ray, cbc, urinalysis, liver and renal function tests, calcium, phosphorus,. ECG, and appropriate radiologic studies including liver/spleen scan and brain scan. Pretreatment immunologic status was evaluated with a skin test battery.

Patients were randomized to one of four treatment arms. Patients in all four treatment arms received radiation therapy beginning on Day 1 of the study. Radiation was given as a split course by administering 3000 cGy (rad) at the ratio of 300 cGy/fraction for ten fractions in 2 weeks. After a 3-week rest, a second course of 3000 cGy in 2 weeks was prescribed, with appropriate blocking to protect critical normal structures such as heart and spinal cord. A posterior spinal cord block was permitted for the second course to shield the spinal cord. Arm 1 utilized levamisole in addition to the radiation therapy, with the drug being administered at a dose of 100 mg/m^2/day (rounded to the nearest 50 mg) on Days 10 and 11, toward the end of the first course of radiotherapy, and thereafter on 2 consecutive days at weekly intervals. During the second course of radiotherapy, levamisole was not begun until the last day of that session. Levamisole administration was then continued for 2 years, or until evidence of progressive tumor growth. Arm 2 utilized doxorubicin as a single dose of 50 mg/m^2 iv on Day 29 (4 weeks from the start of treatment and 17 days after completion of the first course of radiotherapy). In this treatment arm, the second course of radiation was delayed for 3 weeks after the administration of the doxorubicin. Doxorubicin therapy was resumed 3 weeks after the completion of the second course of radiation therapy and was continued at 50 mg/m^2 every 3 weeks to a total dose of 450 mg/m^2. After the completion of the doxorubicin therapy, no further treatment was given on this arm. Arm 3 was a combination of Arms 1 and 2, with therapy given exactly as described in those treatment arms, except that levamisole was not given on the same day as doxorubicin. Arm 4 consisted of radiation therapy alone.

The definitions of response were the standard definitions of the Southwest Oncology Group: *complete response (CR)*—the disappearance of all evidence of disease (confirmatory bronchoscopy was not required) for at least 4 weeks; and *partial response (PR)*—≥ 50% reduction of the sums of the areas of all measurable disease for the same time period. The area of any lesion was considered to be the product of its greatest perpendicular diameters. Survival status was obtained by measuring the time from entry in the study until death. Statistical comparisons of survival were done using the Wilcoxon-Gehan test (17), and survival curves were computed using the method of Kaplan and Meier (18). The two-sided test of significance was applied to all comparisons.

RESULTS

One hundred and twenty-five patients from 31 institutions (one to 25 patients per institution) were registered in the study when it ended in October 1979. Eighteen patients were ineligible because of wrong histology or stage of disease. The remaining 107 patients form the basis of this report.

Response

Of the 107 eligible patients, there were 15 (14%) with CR and 20 (19%) with PR. Stable disease was observed in six (6%) patients.

The CR plus PR rates of the four individual treatment arms varied from 18% to 39% (table 1). When compared simultaneously, these rates are not significantly different ($P = 0.327$). Comparison by chemotherapy, immunotherapy, surgery, and performance status showed no statistically significant difference in response rates or in survival. The overall median duration of response for the 35 patients with CR or PR was 27 weeks. Of these 35 responders, 28 (80%) had relapsed at the time of analysis.

Survival

Median survival of all 107 eligible patients was 42 weeks. Of the 107 patients, 86 (80%) have died. The survival of patients in the combined doxorubicin arms (fig 1) was not significantly different ($P = 0.308$) from that of patients in the combined treatment arms not containing doxorubicin.

The survival of patients in the combined leva-

TABLE 1.—Summary of response according to stratification and treatment-related variables

	No. of patients (%)			
Characteristic	Total	CR/NED*	PR	*P* value†
Total eligible	107 (100)	15 (14)	20 (19)	—
Chemotherapy status				
Doxorubicin	53 (50)	5 (9)	9 (17)	0.242
No doxorubicin	54 (50)	10 (19)	11 (20)	
Immunotherapy status				
Levamisole	53 (50)	7 (13)	8 (15)	0.499
No levamisole	54 (50)	8 (15)	12 (22)	
Treatment arm				
Radiation therapy + levamisole	26 (24)	5 (19)	5 (19)	0.327
Radiation therapy + doxorubicin	26 (24)	3 (12)	6 (23)	
Radiation therapy + levamisole + doxorubicin	27 (25)	2 (7)	3 (11)	
Radiation therapy only	28 (26)	5 (18)	6 (21)	
Surgical status				
Inoperable	90 (84)	12 (13)	19 (21)	0.550
Incomplete resection	17 (16)	3 (18)	1 (6)	
Performance status				
1–4	7 (7)	1 (14)	0 (0)	0.361
5–7	35 (33)	5 (14)	9 (26)	
8–10	65 (61)	9 (14)	11 (17)	

*NED = no evidence of disease.

†Test of CR + PR vs other response for all patients by characteristic.

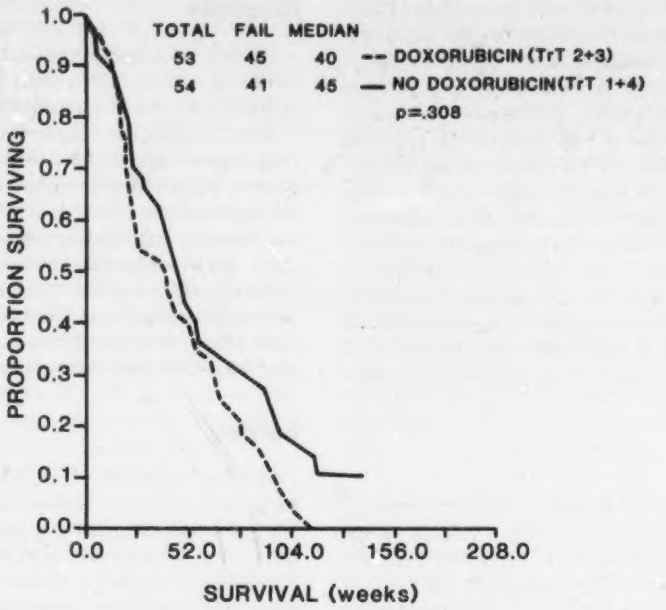

FIGURE 1.—Survival according to chemotherapy status (treatment Arms 2 and 3 vs treatment Arms 1 and 4).

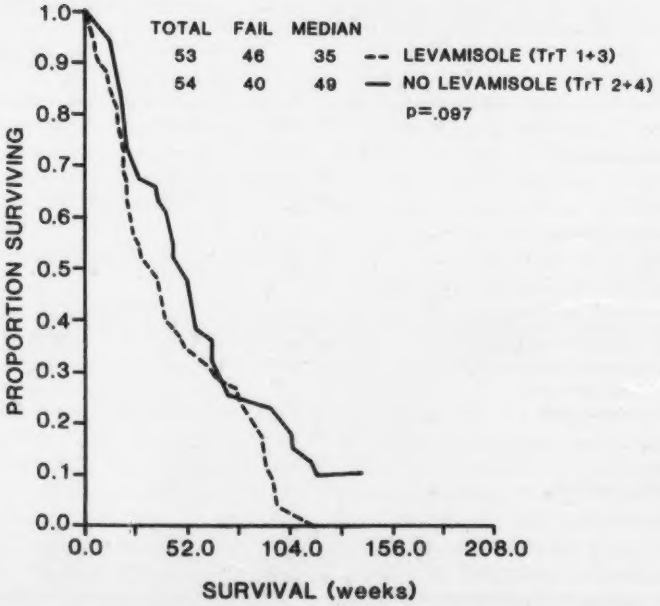

FIGURE 2.—Survival according to immunotherapy status (treatment Arms 1 and 3 vs treatment Arms 2 and 4).

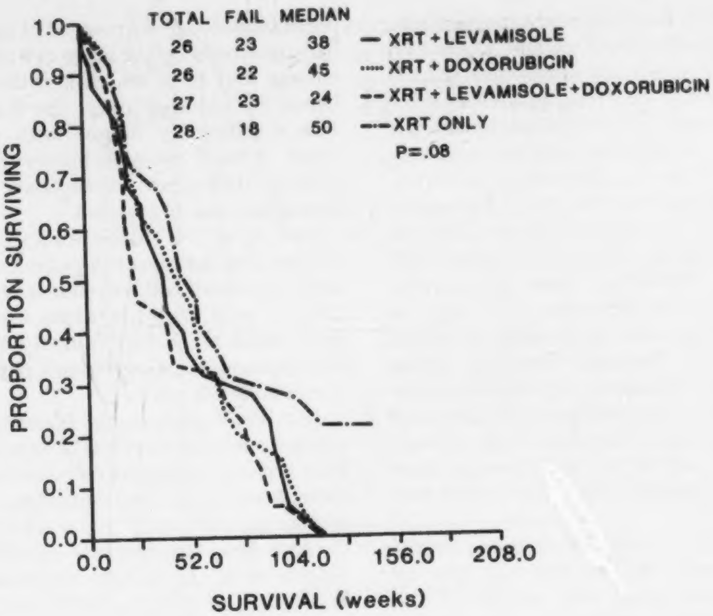

FIGURE 3.—Survival analyzing all 4 treatment arms separately. XRT = radiation therapy. For the 4-sample test, $P = 0.295$. All pairwise comparisons other than that shown above, $P > 0.21$.

misole arms (fig 2) was found to be shorter ($P = 0.097$) than that of patients in the combined arms not containing levamisole. These two survival curves are completely distinct for the first 13–14 months after initiation of treatment.

When all four treatment arms are compared individually (fig 3), patients receiving radiation therapy alone (Arm 4) had the best survival, but none of the differences were statistically significant (lowest $P = 0.08$).

Pretreatment stratification variables had no influence on survival. There are similar numbers of patients with weight loss or supraclavicular metastasis in all treatment arms, but too few to make any meaningful analysis of prognostic significance. As in most studies, the survival of responding (CR and PR) patients (fig 4) was found to be highly significantly longer ($P = 0.002$) than that of nonresponders.

Sites of Failure

Seventeen of the eligible patients have not yet failed. Of the 90 remaining patients, 50 failed in the radiation therapy field only (32 in the primary tumor only, 17 in the primary plus nodes, and one in the primary plus pleura). Eighteen patients failed in the primary tumor and distant sites, two failed peripherally in the lung, and seven failed in distant sites only. In 13 patients the initial site of failure was unknown.

Toxicity

There were two fatal toxic effects: one patient in the radiation therapy plus doxorubicin treatment arm died of thrombocytopenia, and one patient in the radiation-only arm died of radiation pneumonitis. There were five patients with severe toxic effects: two with severe pulmonary effects (one in the radiation-alone arm and one in the levamisole and doxorubicin arm); two with severe nausea and vomiting (one in the levamisole arm and one in the levamisole and doxorubicin arm); and one with severe radiation esophagitis (in the doxorubicin arm). With those exceptions toxicity was mild to moderate. Toxic effects were either absent or mild in 52% of patients receiving levamisole and doxorubicin and in 75%–80% of patients in the other treatment arms.

DISCUSSION

A number of controlled and uncontrolled studies have compared daily fractionated radiation to a split course approach. Some have claimed superiority for the split course approach (19–21), while others have found no statistically significant differences (22,23). However, until recently, no investigator has found the split course approach to be significantly *inferior* to a chronic, daily fractionation scheme, and most investigators have found the split course to be better tolerated by patients (24–31). In a randomized study, Guthrie et al (25) showed statistically superior 1-year survival for 3000-cGy/2 weeks split course compared to 2000-cGy/1 week split course. After 1 year there was no difference, with only one of 97 patients surviving after 24 months. A recent randomized Radiation Therapy Oncology Group (RTOG) study showed statistically significantly worse survival for the split course regimen (5) compared to the continuous course. However, these investigators employed two 2000-cGy (rad)/1 week short radiation courses delivering a total dose of only 4000 cGy.

Although the RTOG demonstrated worse results for split course therapy (5), the 18% CR rate for radiation alone in this study was similar to that produced by their best treatment arm (6000 cGy/6 weeks, continuous radiation). Patients had failure in the primary site alone or with other sites including nodes, pleura, and distant metastasis. The pattern is similar to that reported from the Veterans Lung Study Group Protocol 15 (32) and in the RTOG study (5).

Given the limited effectiveness of radiotherapy alone for squamous cell lung carcinoma with evidence of regional spread, a number of investigators have explored the approach of administering additional chemotherapy to such patients. Early studies involved addition of one or two "short courses" of chemotherapy at the time radiation was delivered, in the hope that this would add to the effectiveness of the radiation by a "potentiating" effect. Numerous studies, including several which involved prospective randomization, demonstrated no evidence of added benefit.

A second approach involved the administration of chronic, daily, or weekly "low-dose" chemotherapy during or after administration of radiation therapy; again, the results of randomized, controlled studies showed no advantage over radiotherapy alone (6–8).

More recently, a few studies have been done in which radiotherapy is given in conjunction with an approach involving the administration of high-dose, intermittent chemotherapy, with spaced intervals off therapy to allow for host immunologic recovery and reconstitution. Bergsagel et al (9) reported a randomized study of patients treated in this fashion, comparing radiation alone to radiation plus cyclophosphamide. In a group of 74 patients, 56 of whom had squamous cell or large cell undifferentiated carcinoma and 18 of whom had their diagnosis established by cytology only, the 3–6 months' survival was significantly longer with combination treatment. After 6 months, however, there was no significant difference: metastases seemed to be delayed, but not prevented.

Carr et al (22) studied radiation alone versus radiation plus 5-FU in 188 patients. Of those patients with non-small cell carcinoma of the lung, 66 were treated with radiation alone and 64 were treated with radiation therapy plus 5-FU (22). Six percent of 31 squamous cell carcinoma patients in each group survived for 3 years.

In a small pilot study, Hansen et al (10) demonstrated a median survival of 49 weeks in ten patients with limited squamous cell carcinoma who received cyclophosphamide and methotrexate followed by radiotherapy (5000 cGy in 6 weeks), and then more of the two-drug intermittent combination. However, Shields et al (11) of the Veterans Administration, in a study of patients with localized disease who received cyclophosphamide and methotrexate in an alternating fashion every 5 weeks after surgery, failed to demonstrate an advantage for the combined approach over surgery alone. Samuels et al (12) gave a combined approach with vincristine, bleomycin, and methotrexate prior to radiation of the primary tumor given as a split course, and then further intermittent chemotherapy was given to those patients who appeared to benefit: MST was 42 weeks in 27 patients, with 21 of the 27 patients having squamous cell histology. Only those patients with squamous cell histology demonstrated objective tumor regression in this series, and this group of responders had an MST of 70+ weeks, as opposed to 26 weeks for the nonresponders. A similar difference, but without such striking prolongation among responders, has been observed between those patients with evidence of objective regression after radiotherapy alone, and those without such evidence. This prolongation of survival in responders was observed in the current study (fig 4). However, chemotherapy, as employed in the current study, did not contribute to either response or survival. Continued followup of study patients is planned. It is highly unlikely that any treatment arm will become better than radiation alone.

Adjuvant immunotherapy in limited squamous cell lung cancer is also controversial. A survival advantage in small series was demonstrated for patients receiving intrapleural BCG at the time of surgery (33) and for patients receiving BCG by the Heaf gun technique (15) at regular intervals after radiotherapy. In addition, patients randomized to receive

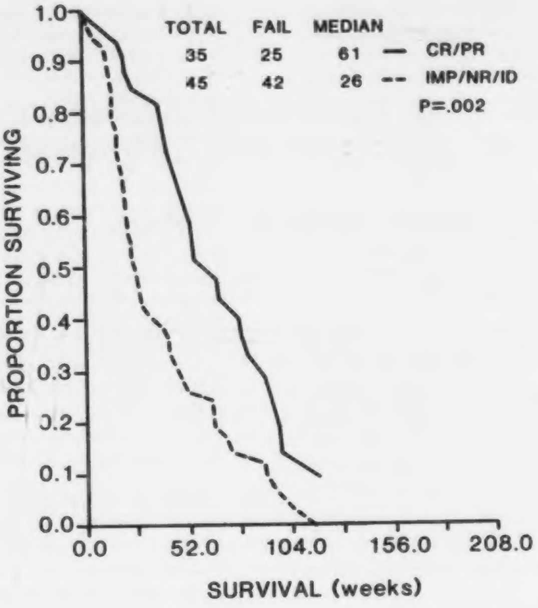

FIGURE 4.—Survival comparison of response-eligible patients. IMP = improved, NR = no response, ID = increasing disease.

levamisole (50 mg three times a day for 3 days every 2 weeks) after surgical resection appeared to have a significantly lower early recurrence rate, compared to concurrent controls (16). Though not statistically significant, patients receiving levamisole in the current study had a worse survival.

If the chemotherapy and/or immunotherapy were to produce any detectable benefit, it should be demonstrable regardless of the type of radiation employed, as long as the radiation was consistent in all treatment arms. Although the number of patients entered in each arm was relatively small, the superior survival achieved by patients treated with radiation alone is not likely to be exceeded by the inclusion of additional patients.

CONCLUSION

In this four-arm randomized study neither doxorubicin chemotherapy nor levamisole immunotherapy contributed to survival or local control, when compared to patients treated with radiation alone.

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Combination Chemotherapy for Patients With Relapsed Malignant Lymphoma Using Methyl-GAG and Teniposide (VM-26)[1,2,3]

Raymond P. Warrell, Jr,* David J. Straus, and Charles W. Young[4,5]

SUMMARY

We treated 45 patients with advanced malignant lymphoma, using a combination of methyl-GAG and teniposide (VM-26). All patients had received extensive prior treatment with combination chemotherapy with or without irradiation. Both methyl-GAG (600 mg/m^2) and VM-26 (100 mg/m^2) were administered on Days 1 and 8 of the treatment protocol and, in responding patients, every 2 weeks thereafter. Partial responses occurred in six of 12 patients with Hodgkin's disease and in ten of 31 patients with non-Hodgkin's lymphoma. The median duration of response for all patients was 3½ months (range, 1½–11). There were moderate toxic effects, including nausea, myalgia, weakness, and myelosuppression. Relative to our recent experience with methyl-GAG as a single agent, the addition of VM-26 to methyl-GAG did not produce a superior rate or duration of response in similar, heavily pretreated patient populations with malignant lymphoma; however, the combination caused considerably more myelotoxicity. We conclude that the use of VM-26 with methyl-GAG in this dose schedule offers no advantage over single-agent therapy. Methyl-GAG, when administered on a biweekly schedule, is effective and well-tolerated. This agent should be considered for incorporation into chemotherapy protocols for the therapy of previously untreated patients with malignant lymphoma.

[Cancer Treat Rep 66:1121–1125, 1982]

Despite extraordinary progress in the treatment of malignant lymphoma, the prognosis for most patients who relapse from aggressive combined-modality treatment remains poor (1,2). In an effort to improve the salvage treatment of these patients, we developed a protocol that utilized a combination of methyl-GAG and teniposide (VM-26) for patients whose disease had progressed following first-line chemotherapy/radiotherapy.

The choice of these agents reflected several considerations: (*a*) both drugs are active as single agents in lymphoma, with response rates ranging from 20% to 40% (3,4); (*b*) the putative mechanisms of action for these drugs differ both from each other and from other conventional chemotherapeutic agents; (*c*) the drugs do not have overlapping toxic effects; and (*d*) methyl-GAG and etoposide (VP-16-213, a congener of VM-26) are a synergistic combination in murine L1210 leukemia (J. Burchenal, personal communication). Although both VP-16-213 and VM-26 are active in lymphoma (4,5), certain clinical data suggested that VM-26 was the more active compound (6); however, no direct comparison of these agents has ever been made.

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[1] Received Sept 11, 1981; revised Dec 7, 1981; accepted Dec 17, 1981.

[2] Supported in part by Public Health Service grant CA-05826 and contract N01-CM-57043 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Methyl-GAG and VM-26 were supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4] Developmental Chemotherapy (*R. P. Warrell, Jr*, and *C. W. Young*) and Hematology/Lymphoma Services (*D. J. Straus*), Department of Medicine, Memorial Sloan-Kettering Cancer Center and the Cornell University Medical College, New York, NY.

[5] We are indebted to Ms. Catherine Cassidy for assistance with data collection and Ms. Shelly Hantman for preparation of the manuscript.

**Reprint requests to:* Dr. R. P. Warrell, Jr, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

Immediately following our evaluation of methyl-GAG as a single agent (3), we initiated a trial in which methyl-GAG and VM-26 were used in combination for the treatment of patients with relapsed disease. This report details the results of that investigation.

PATIENTS AND METHODS

Forty-five patients with Hodgkin's disease or non-Hodgkin's lymphoma who had failed conventional therapy were entered in this study. The treatment protocol required histologic confirmation of disease, bidimensionally measurable disease parameters, Karnofsky performance status ≥ 50, adequate renal and hepatic functions (serum bilirubin and creatinine ≤ 1.5 mg/dl), and adequate bone marrow reserve (wbc count $\geq 3500/\text{mm}^3$ and platelet count $\geq 120{,}000/\text{mm}^3$). Patients had not received irradiation or chemotherapy within 3 weeks preceding entry, nor had they previously received either methyl-GAG or VM-26. Pretreatment evaluation included: history and physical examination; hemoglobin and wbc and platelet counts; serum biochemical screening profile; and chest roentgenogram. Additional procedures were performed as needed to assess the extent of disease. All patients gave signed, informed consent, and the study was approved in advance by the Clinical Investigations Committee of the Memorial Sloan-Kettering Cancer Center.

Response Criteria

Complete response was defined as the disappearance of all evidence of disease and normalization of all biochemical parameters for > 4 weeks. In addition, a complete response required restaging, which included, as a minimum, bone marrow biopsy and abdominal computed tomographic scan. Partial response was defined as a > 50% decrease in the sum of the products of the longest perpendicular diameters of all measurable lesions, without the appearance of new lesions for at least 4 weeks. Tumor regressions of < 50% or < 4 weeks duration were recorded as failures. Duration of response was defined from the date on which a > 50% response was observed to the date of disease progression.

Method of Administration

After reconstitution with normal saline, the prescribed dose of methyl-GAG was further diluted in 250 ml of 5% dextrose solution and was infused iv over 60–90 minutes. VM-26 was diluted in 100 ml of 5% dextrose solution and was administered iv over 30 minutes. The dose of methyl-GAG was held constant at 600 mg/m^2. The initial dose of VM-26 was 100 mg/m^2; this dose was escalated by increments of 20 mg/m^2 in the absence of myelotoxicity (wbc count $< 3000/\text{mm}^3$ or platelet count $< 100{,}000/\text{mm}^3$). Both drugs were administered on Days 1 and 8 of the treatment protocol. Patients who did not manifest progressive disease during this initial therapy then received both drugs every 14 days. Patients were considered adequately treated and evaluable for response after receiving two doses of each drug, with or without toxic effects.

RESULTS

The pretreatment characteristics of the patients who received this therapy are shown in table 1. All patients had received extensive combination chemotherapy prior to entry in this trial. Thirty-seven patients (80%) had also received radiation therapy. Forty-three patients (93%) had stage IV disease (Ann Arbor classification). Twenty-seven patients (61%) had liver, lung, or bone marrow involved with lymphoma. Other extranodal sites involved included: pleura (seven patients); skin and cortical bone (six each); stomach, colon, and pericardium (two each); and lacrimal gland, testis, and thyroid (one each).

Therapeutic Effects

Of the 45 patients entered in the study, 43 are evaluable for response; two patients received only a single dose of each drug. One of these patients showed regression of her skin disease but failed to return for followup treatment, and the other had complete disappearance of pain due to tumor involving the sacral plexus but subsequently sustained a fatal pulmonary embolus. As shown in table 2, 16 of 43 patients (37%) with a variety of histologic subtypes responded. After careful restaging, none of these responses was believed to be complete, although in some patients the residual abnormalities were minimal (eg, unexplained elevation in alkaline phosphatase and persistent leukopenia without residual lymphoma demonstrable on bone marrow biopsy).

Six patients in this trial failed to return for followup therapy. With the one exception previously cited, the other five patients had experienced major regressions of disease. Patients were contacted to ascertain their reasons for noncompliance. Two patients refused treatment because of toxic effects (neuropathy and dysesthesia, discussed below), and the other three failed to return for psychosocial reasons. These patients were therefore removed from the study at periods ranging from 1 to 7 months after a > 50% disease response had been observed

TABLE 1.—Pretreatment characteristics of patients treated with methyl-GAG/VM-26 combination

Characteristic	No. of patients
Histologic subtypes†	
Hodgkin's disease	12
DHL	15
DPDL	7
NPDL	7
NHL	1
DML	1
Major sites of extranodal involvement	
Bone marrow	11
Lung/pleura	17/7
Liver	4
Median	
Age in yrs (range): 51 (20–71)	
Karnofsky performance status (range): 60 (50–80)	
No. of prior chemotherapy regimens* (range): 2 (1–6)	
No. of prior chemotherapeutic drugs (range): 5 (3–12)	

*A regimen of chemotherapy was defined as 1 drug (an investigational agent) used alone or several drugs used in combination (eg, cyclophosphamide, doxorubicin, vincristine, and prednisone [CHOP]) for at least 4 wks.

†DHL = diffuse histiocytic; DPDL = diffuse poorly differentiated lymphocytic; NPDL = nodular poorly differentiated lymphocytic; NHL = nodular histiocytic; and DML = diffuse mixed lymphocytic-histiocytic.

(table 2). The median duration of response for the remaining 11 patients was 3½ months (range, 1½–11).

Toxicity

Toxic effects related to the use of this combination are presented in table 3. All patients experienced mild facial paresthesia during the infusion of methyl-GAG. Nausea was generally confined to the period of drug infusions. A syndrome of myalgia and muscular weakness was again noted in this trial; in each instance it was related to the dose of methyl-GAG on Day 8 and it usually resolved within 10–14 days. This syndrome seemed especially common in debilitated patients with lower performance status. Three patients developed painful, subcutaneous nodules on the lower extremities. Biopsy of these lesions revealed a perivascular infiltration of inflammatory cells. These lesions were also related to the Day-8 dose of methyl-GAG and resolved in 3–4 weeks. One patient developed an acute, severe peripheral neu-

TABLE 2.—Response rates of patients with malignant lymphoma treated with methyl-GAG/VM-26 combination

Histology*	No. of patients		Response rate (%)	Duration of response (mos)
	Responding	Evaluable		
Hodgkin's disease	6	12	50	9+, 9+, 7,† 5+, 2,† 2†
DHL	5	15	33	2, 2, 1½, 1½,† 1†
DPDL	2	7	29	11, 3½
NPDL	2	7	29	5, 1½
DML	1	1	—	5+
NHL	0	1	—	—

*For definitions, *see* table 1.

†Inadequate followup (*see* text).

TABLE 3.—Toxicity of methyl-GAG/VM-26 combination in 43 patients

Toxic effect	No. of patients	%
Facial paresthesia	43	100
Vomiting	11	24
Myalgia	9	20
Muscular weakness	7	16
Vasculitis	3	7
Chills during drug infusions	2	4
Skin rash	2	4
Peripheral neuropathy/dysesthesia	1/1	2
Leukopenia		
Wbc count (cells/mm^3)		
2000–2999	10	
1000–1999	8	
< 999	6	
Thrombocytopenia		
Platelet count (cells/mm^3)		
50,000–99,999	7	
< 50,000	6	

ropathy involving both sensory and motor functions. Because this patient had achieved a marked antitumor response, we re-treated her with VM-26 alone after her symptoms improved. She then experienced marked worsening of her neuropathy without any further antitumor response and was removed from the study. Thus, it appears that this effect was caused by VM-26. Two patients developed fever and chills during infusions of VM-26. This effect was considered to be intolerable in one patient and his response has been maintained with methyl-GAG as a single agent.

The most significant toxic effect of this combination was myelosuppression. Following the Day-8 dose of methyl-GAG and VM-26, eight patients (18%) required hospitalization because of fever and granulocytopenia. Sepsis related to myelosuppression resulted in the death of one patient with borderline cardiac function. The median wbc count nadir for all patients was 2700/mm^3 (range, 100–18,500); the median platelet count nadir was 153,000/mm^3 (range, 10,000–441,000).

DISCUSSION

Using VM-26 and methyl-GAG in combination, we achieved an overall response rate of 37% in our patients with advanced malignant lymphoma. Although this chemotherapy program was generally well-tolerated, the rate of response was not superior to that observed with the use of methyl-GAG as a single agent. In a trial that immediately preceded the current study, 37% of lymphoma patients responded to methyl-GAG; response rates in individual histologic subtypes were also similar (3). This lack of improvement cannot be attributed to an imbalance of important prognostic features between the two patient populations. All patients in both trials had received extensive prior therapy; however, the requirement for normal hematologic counts in the current study recruited patients with somewhat better performance status who had been treated with fewer drug regimens than in the single-agent trial (3). Thus, these factors might have been expected to favor results for the combination.

It is conceivable that an unrecognized adverse interaction between methyl-GAG and VM-26 was responsible for the lack of increased efficacy of the combination. Therapeutic synergy between the epipodophyllotoxins and other chemotherapeutic agents can be schedule-dependent (7). Roberts et al (8) recently noted that synergy between VM-26 and BCNU (carmustine) in L1210 leukemia which occurs when both drugs are given on Day 1 is lost when administration of VM-26 is delayed until 4 days after the injection of BCNU. Other investigators have also noted unexpectedly low response rates with VM-26 combinations. Subsequent to the report of Chiuten et al (4), the Eastern Cooperative Oncology Group (9) combined VM-26 with hexamethylmelamine plus prednisone and with cisplatin plus prednisone in two separate studies; the overall response rates were a disappointing 8% and 19%, respectively. Lachant et al (10) combined VM-26 with procarbazine, dexamethasone, and methotrexate, with initially favorable results; however, more extensive evaluation revealed an unimpressive rate of response, and the combination was not recommended for further evaluation. Certain clinical data in patients with small cell lung cancer suggested that VP-16-213, a congener of VM-26, was more effective when administered in divided doses (11). Furthermore, European investigators (6,12) reported somewhat higher response rates, using daily or divided-dose schedules of VM-26 in their patients with lymphoma. In view of the disappointing results from these latest trials, there seems little indication for further study of VM-26 using weekly or biweekly dose schedules in heavily pretreated patients.

From a clinical standpoint, we believe methyl-GAG was clearly the more important drug for patients who responded to the combination. It is clear, however, that weekly administration of this agent causes excessive toxicity. Adverse reactions to methyl-GAG in this study were almost entirely related to drug administration on Days 1 and 8. Conversely, the biweekly schedule was exceptionally well-tolerated. Recent pharmacokinetic data (13) have shown excretion of unchanged drug up to 14 days following iv administration. Thus, it seems that toxicity is associated with delayed excretion or drug accumulation during the terminal phase.

We have also noted a dose-schedule relationship in certain patients who responded to methyl-GAG as a single agent (3). In these patients, reducing the dose to ≤ 400 mg/m^2 (in three patients) or lengthening the interval between courses to 3 weeks (in four patients) resulted in regrowth of disease. Each patient again responded following a dose increase to 600 mg/m^2 and/or a decrease in the treatment interval to 2 weeks. Therefore, we believe a dose of 600 mg/m^2 administered every 10–14 days optimizes the therapeutic index for this drug. In view of its efficacy, minimal toxicity in the schedule described, lack of significant myelosuppression, and unique mechanism of action, methyl-GAG merits advancement into front-line combination chemotherapy programs for patients with malignant lymphoma.

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Phase I Trial of Aclacinomycin A[1,2]

David A. Van Echo,[3,*] Margaret Y. Whitacre,[3] Joseph Aisner,[3] Mark M. Applefeld,[4] and Peter H. Wiernik[3]

SUMMARY

Aclacinomycin A (ACM-A), an anthracycline analog, was given to 17 patients with solid tumors and to one patient with multiple myeloma, in a phase I clinical trial. A single dose of 60–120 mg/m² was given every 3 weeks. Dose-limiting toxicity was myelosuppression, especially thrombocytopenia. Granulocytopenia was variable and did not always recover by Day 21 in time for the next ACM-A treatment. Other toxic effects were nausea, vomiting, urticaria, and elevation of hepatic enzymes. Alopecia was not a side effect, even in patients receiving multiple courses of ACM-A. Nine patients were monitored with 24-hour continuous ECG recordings (Holter) on 19 ACM-A treatment days. The incidence of premature atrial and ventricular beats was significantly increased following ACM-A administration. In addition, one patient developed episodes of high-degree atrioventricular block and complete heart block after each of four ACM-A doses, necessitating the insertion of a pacemaker. No antitumor responses were seen in the ten patients who had measurable disease and who had received two or more courses of ACM-A. The recommended doses for solid tumor phase II studies are 100 mg/m² as a single dose every 4 weeks for patients with high performance status and minimal prior chemotherapy and 60 mg/m² every 4 weeks for all other patients. Until the acute cardiac effects of ACM-A are further understood, we recommend that all patients receiving ACM-A be monitored by ECG recordings.

[Cancer Treat Rep 66:1127–1132, 1982]

Aclacinomycin A (ACM-A) is a new yellow anthracycline antibiotic isolated from the culture of *Streptomyces galilaecus MA 144-M1* by Oki et al in 1975 (1). It has a structure (fig 1), mechanism of action, pharmacokinetics, and toxicity different from doxorubicin (ADM) and other anthracycline compounds. In general, the animal antitumor activity of ACM-A is comparable to that of daunorubicin (DNR) but somewhat less than that of ADM; in contrast to the other anthracyclines, ACM-A is also active by the oral route (2). The optimal dose both clinically and preclinically is approximately twice that of ADM. The anthracyclines are potent inhibitors of DNA synthesis through their ability to intercalate between adjacent nucleotides in native DNA, which results in significant alterations in secondary structure, sensitivity to nucleases, and template activity. ADM and DNR inhibit synthesis of DNA, cytoplasmic RNA, and nucleolar RNA at equal concentrations (3). In contrast, although ACM-A inhibits DNA synthesis, it preferentially inhibits cellular RNA synthesis at concentrations sixfold to sevenfold lower than those required to inhibit DNA

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[1] Received July 16, 1981; revised Nov 2, 1981; accepted Nov 23, 1981.

[2] Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Baltimore Cancer Research Center, University of Maryland Hospital.

[4] Division of Cardiology, Department of Medicine, University of Maryland Hospital, School of Medicine, Baltimore.

[*] *Reprint requests to:* David A. Van Echo, MD, Baltimore Cancer Research Center, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

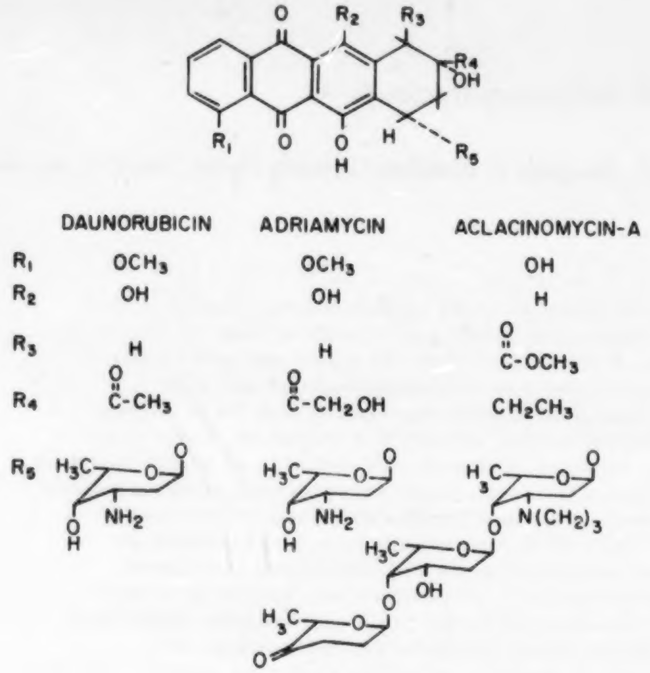

FIGURE 1.—Structures of DNR, ADM, and ACM-A.

synthesis, and it inhibits nucleolar RNA synthesis at concentrations 170-fold to 1250-fold lower than those necessary to inhibit DNA synthesis (4). It is most active in the G_1-S transition and late S-G_2 phases of mitosis (2). Work in our laboratory by Egorin et al (5) indicates that cellular uptake of ACM-A is more rapid than that of ADM or DNR and that ACM-A preferentially accumulates in the cytoplasm of cells; ADM and DNR accumulate in the nucleus. Studies of acute and subacute toxicity in rodents have shown that ACM-A causes significantly less quantitative and qualitative changes in cardiac function, arrhythmia induction, and ultrastructural myocardial damage than does ADM or DNR (2,6).

Phase I and II clinical trials of > 200 patients have been completed in Japan.[5] Virtually all patients had solid tumors or lymphoma. Three dose schedules of iv administration were studied: one dose per day × 4–10 days, one dose two or three times per week, and one dose every 2–3 weeks. Gastrointestinal toxicity was the most frequent adverse reaction (50%), irrespective of dose schedule, but was resolved within 24 hours. Leukopenia (35%), thrombocytopenia (20%), anemia (13%), and hepatic enzyme changes (10%) were dose-dependent and dose-limiting but reversible within 2–3 weeks. Stomatitis and alopecia were infrequent (about 1%). Clinically insignificant transient ECG changes were detected in ten patients. The followup period was too short and the number of patients with dose accumulations of ACM-A comparable to those of ADM or DNR, which cause congestive heat failure, was too small to make any assessment of chronic cardiac toxicity due to ACM-A. Objective tumor responses seen in a spectrum of patients suggest that ACM-A will mimic the activity of ADM, ie, in breast and ovarian cancer and lymphoma. The number of patients with leukemia treated was too small to allow comment on any activity in this disease. The recommended dose of ACM-A for phase II trials is 17 mg/m²/day × 7 every 3 weeks or 100–120 mg/m² once every 3 weeks.

To compare the toxic effects of ACM-A with those of ADM it was decided to administer a single dose every 3 weeks and to choose an initial dose 50% of that recommended from the Japanese trials.

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[5] Material on file at the Investigational Drug Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD. Presented at the Decision Network II Meeting, Sept 25, 1978.

MATERIALS AND METHODS

Patient Selection and Evaluation

Eighteen patients (nine males and nine females) with a median age of 52 years (range, 30–70) and a median performance status of 60% (range, 20%–70%) received one or more courses of ACM-A (total, 34 courses). Seventeen patients had solid tumors and one had multiple myeloma (table 1). Criteria for entry in the study required histologic proof of a malignant disease unresponsive to conventional therapy, no chemotherapy or radiotherapy for at least 4 weeks prior to entry (8 weeks in the case of nitrosoureas), and a minimum life expectancy of 6 weeks. Patients were expected to have acceptable blood cell counts (wbc count ≥ 4000/μl; platelet count ≥ 100,000/μl) and normal hepatic and renal functions. Measurable disease was not required. Written informed consent was obtained from each patient. All patients had received extensive prior chemotherapy, including six who had received nitrosoureas or mitomycin. Twelve patients had received both radiotherapy and chemotherapy.

TABLE 1.—Tumor types in patients treated with ACM-A

Type of disease	No. of patients	No. evaluable for response*
Soft tissue sarcoma		
Fibrosarcoma	1	1
Clear cell sarcoma	1	1
Malignant fibrous histiocytoma	1	1
Breast cancer	3	2
Colon cancer	2	1
Renal cancer	2	1
Melanoma	2	2
Bladder cancer	1	
Esophageal cancer	1	
Oat cell carcinoma, lung	1	
Ovarian cancer	1	—
Uterine cancer	1	—
Multiple myeloma	1	1
Totals	18	10

*Criteria for evaluation for response included measurable disease and at least 2 courses of ACM-A.

Baseline studies performed before treatment included: hematocrit; wbc count with differential count, platelet count, urinalysis, serum electrolytes, glucose, BUN, calcium, phosphorus, uric acid, creatinine, bilirubin, albumin, total protein, alkaline phosphatase, SGOT, LDH, prothrombin, partial thromboplastin time, and fibrinogen. During therapy, wbc and platelet counts and coagulation and biochemical tests were repeated weekly.

Drug and Schedule of Administration

ACM-A was supplied by the Investigational Drug Branch of the National Cancer Institute (Bethesda, MD) as a formulation in 5- or 10-ml ampules containing 0.5 mg/ml of the free base of ACM-A. The desired dose was added to 10–20 ml of 5% dextrose in water for administration. Since the supplied solutions did not contain antibacterial preservatives, the ACM-A was given within 4 hours after preparation. In this study, each dose of drug was administered iv over 10 minutes.

Patients received ACM-A on an inpatient or outpatient basis, depending on their overall medical condition. Pulse, temperature, and blood pressure were recorded prior to administration and at 15–30-minute intervals thereafter as indicated, for 2–4 hours.

One course of treatment consisted of a single dose of ACM-A. Courses were repeated every 3 weeks (table 2). At least two patients were treated at each nontoxic dose level and were observed for 2 weeks before patients were treated at a higher dose. Five or six patients were treated at any level at which reversible toxic effects were observed. One of the 18 patients, who had received two courses of ACM-A at a single dose level without undue toxic effects and had tumor stabilization and subjective improvement, received two additional courses at the next highest dose level.

TABLE 2.—Dose increments of ACM-A and No. of patients treated at each level

Dose (μg/m²)	Total No. of patients	No. of patients who received— 1 course	2 courses	3 courses	4 courses
60	2	2	1	0	0
90	7	7	3	0	0
100	6	6	3	0	0
120	5	5	4	1	1

RESULTS

Hematologic Toxicity

All 18 patients are evaluable for nonhematologic toxicity and 17 are evaluable for hematologic toxicity (one patient had a bone marrow infiltrated with multiple myeloma and is not considered evaluable for hematologic toxicity). Hematologic side effects are listed in table 3. Dose-related and dose-limiting toxicity was myelosuppression, which occurred in 15 patients (88%). Leukopenia (wbc count < 4000/µl) was absent at the 60-mg/m² dose level and was moderate to severe at the 90–100-mg/m² dose level. At the 90-mg/m² dose level, four of seven patients developed a modest leukopenia, with return to pretreatment levels by Day 4. At the 100-mg/m² dose level the leukopenia was more pronounced (median, 2700 cells/µl; range, 600–5500), but recovery was still within 24 days. However, at the 120-mg/m² dose level the nadir wbc count was not only more severe (median, 1500/µl; range, 600–3800), but recovery to 4000/µl required > 28 days.

Granulocyte count nadirs usually coincided with wbc count nadirs but did not precisely follow the trend of the wbc count. It was not uncommon to observe the granulocyte count nadir before the wbc count had begun to decrease significantly or, as was more likely the case, to observe that the granulocyte count continued to decrease after the wbc count had returned to the normal range. This is reflected in the wider range of "median days to nadir" and later "median days to recovery" for granulocyte counts compared to wbc counts seen in table 3.

Significant thrombocytopenia occurred at each dose level > 60 mg/m². The median day to nadir was earlier (9–10 days) for platelet counts than for wbc counts (14–20 days), and recovery was accomplished sooner. Three patients with platelet counts < 20,000/µl received prophylactic platelet transfusions. No patient developed any hemorrhagic manifestations while in this study. The thrombocytopenia and leukopenia observed did not appear to be cumulative. Since the majority of patients (15 of 18) had received prior radiotherapy it is not possible to determine if this modality of treatment predisposed to myelosuppression with subsequent ACM-A exposure. Five evaluable patients had previously received either nitrosoureas or mitomycin. Four of these patients had platelet counts between 10,000 and 30,000/µl and only one of the five had a wbc count below the median nadir for the other patients treated at that ACM-A dose level. It would appear that prior nitrosourea or mitomycin therapy may contribute to the development of significant thrombocytopenia with subsequent ACM-A exposure.

Nonhematologic Toxicity

Nonhematologic toxicity was very similar to that produced by other anthracyclines. Nausea and vomiting unresponsive to conventional antiemetics occurred in all patients (100%). One patient (4%) at the 60-mg/m² dose level developed urticaria along the vein in which ACM-A was being administered. The patient had received several prior courses of ADM and one prior course of ACM-A before developing this symptom. The urticaria subsided after

TABLE 3.—Hematologic toxicity

	Dose (mg/m²)*			
	60	90	100	120
No. of patients	2	7	6	5
No. of evaluable courses	3	8	6	10
Median count nadir (cells × 10³/µl)				
Wbcs	> 4.0	3.1 (1.2–20.0)	2.7 (0.6–5.5)	1.5 (0.6–3.8)
Polymorphonuclear leukocytes	> 1.5	2.2 (0.2–6.3)	1.5 (0.5–2.3)	0.5 (0.1–2.0)
Platelets	> 200	67 (10–300)	60 (19–600)	56 (12–298)
Median days to count nadir				
Wbcs	—	14 (9–21)	20 (16–21)	16 (9–21)
Polymorphonuclear leukocytes	—	16 (6–24)	21 (11–24)	16 (14–23)
Platelets	—	9 (6–14)	10 (7–13)	10 (6–21)
Median days to count recovery†				
Wbcs	—	24 (19–29)	24 (21–32)	28 (21–34)
Polymorphonuclear leukocytes	—	28 (19–29)	28 (24–32)	28 (21–29)
Platelets	—	15 (12–34)	15 (13–17)	21 (16–29)

*Nos. in parentheses = range.

†Criteria for count recovery were: wbc count ≥ 4.0 × 10³/µl; polymorphonuclear leukocyte count, 1.5 × 10³/µl; and platelet count, 100 × 10³/µl.

the termination of the ACM-A infusion and was not accompanied by any other allergic phenomena. One patient (4%) at the 90-mg/m^2 dose level had a transient increase in SGOT to twice normal value during each of two ACM-A courses. The increase was maximal at 10–14 days and returned to normal by the next treatment. Seven patients (39%) complained of a "flushed feeling" and became diaphoretic for a 15–30-minute period 2–4 hours after ACM-A administration. None of the above nonhematologic symptoms appeared to be dose-related with respect to frequency or severity. Alopecia was not graded in a consistent fashion in this study, but those patients who already had alopecia from prior therapy had begun to have return of their hair by the time the ACM-A therapy was discontinued. Of the nine patients evaluable for alopecia at the time of entry in this study, none experienced total alopecia and most had only a slight loss of hair even after two or more courses of ACM-A administration.

Acute Cardiac Toxicity

The potential of ACM-A to produce acute cardiac arrythmias was assessed in nine patients. Five of these monitored patients had received prior ADM therapy, with a median cumulative dose of 439 mg/m^2 (range, 189–777). The time from last ADM exposure was a median of 24 weeks (range, 15–40).

Nineteen 24-hour continuous ECG recordings (Holter) were obtained in nine patients. During the period of monitoring, patients recorded all symptoms and activities. Following a period of 2–19 hours (median, 3) of control recordings, ACM-A was infused and monitoring was continued for 3–20 hours (median, 17). Changes in the frequency of premature atrial and ventricular ectopic beats in these patients are noted in table 4. These differences are significant. In reviewing the individual recordings there was a tendency for ventricular ectopic beats to become multifocal following drug administration. There were two patients in whom isolated couplets of ventricular beats were observed after ACM-A was infused. There were no instances of ventricular tachycardia. High degrees of atrioventricular block (Mobitz 2, 2–4:1) and complete heart block with junctional and idioventricular escape rhythms were noted in one patient. This patient was a 40-year-old man with no known cardiovascular disease and no prior anthracycline exposure. These rhythm disturbances appeared 1–5 hours after ACM-A infusion on each of three occasions and necessitated the insertion of a permanent ventricular pacemaker prior to his fourth course of the drug. Unfortunately, his tumor became unresponsive to chemotherapy after this course of ACM-A and he died 3 months later.

Therapeutic Response

Ten of the 18 patients are considered evaluable for an antitumor response (table 1). No patient achieved any objective tumor shrinkage, although several patients remained stable for two, three, or four courses.

DISCUSSION

The results of this study indicate that ACM-A has tolerable toxicity. Because of variable granulocytopenia, ACM-A as a single dose, should not be given any less frequently than every 28 days. The dose-related and dose-limiting toxicity was myelosuppression, especially thrombocytopenia. Other toxic effects were nausea, vomiting, urticaria, and increase of hepatic enzymes. Alopecia was not a conspicuous side effect even in patients receiving ACM-A at the highest dose for 3–5 months.

The results of this phase I trial are comparable to those of a previously published report by Ogawa et al (7). The patients in that study received ACM-A in a variety of schedules at a dose of 0.6–4.0 mg/kg course (approximately 18–140 mg/m^2). Hema-

TABLE 4.—Frequency of dysrhythmias in 9 patients having continuous ECG recordings prior to, during, and after ACM-A therapy

	No premature beats		Premature beats
	No. of patients	No. of studies	Range/hr (mean)
Before therapy			
Premature atrial beats	8	12	0–2.5 (0.3)
Premature ventricular beats	6	11	0–56 (4.6)
After therapy			
Premature atrial beats	4	5	0–32.9 (3.0)
Premature ventricular beats	4	5	0–265 (19.1)

tologic toxicity was dose-related and dose-limiting, with blood cell count nadirs and time to recovery identical to those of our study. Cardiac toxicity was absent in that study, but the authors do not specify the types of ECG monitoring that were done. It is highly likely that the dysrhythmias we observed would have been missed if continuous ECG recordings had not been done.

ACM-A does have the potential to produce cardiac dysrhythmias in a high proportion of carefully monitored patients. These are usually insignificant and are of no clinical importance. However, one patient in this study developed high degrees of atrioventricular block and, eventually, complete heart block, with symptoms of dizziness and weakness temporally related to ACM-A administration. It is not known what role the emesis induced by ACM-A administration may have played in the production of any of these dysrhythmias, since this was frequently a coinciding problem. Catecholamines were not measured in the patients in this study, so it is not clear whether ACM-A has the potential to elevate this known arrythmia-producing hormone. Because of the small sample size, cardiac observations in this study require verification by larger phase II studies, which are currently underway in many institutions. Until more is understood concerning the arrhythmogenic potential of ACM-A, we recommend that patients receiving ACM-A have continuous cardiac monitoring.

ACM-A is an attractive compound for further clinical trial. It has reproducible and reversible toxic effects in man. The present phase I study indicates that, for patients with high performance status and minimal prior chemotherapy, the dose for phase II studies of ACM-A should be 100 mg/m^2 as a single dose given every 4 weeks and, for all other patients, 60 mg/m^2 every 4 weeks.

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5-FU and Allopurinol: Toxicity Modulation and Phase II Results in Colon Cancer[1,2]

Joan F. Kroener,* Fred Saleh, and Stephen B. Howell[3]

SUMMARY

Concurrent administration of allopurinol allows escalation of 5-FU doses in man when 5-FU is given by continuous infusion for 5 days. Forty-nine patients received 81 courses of treatment with 5-FU and allopurinol in phase I and II trials. The dose-limiting toxicity was mucositis; marrow toxicity was mild. Neurotoxicity, possibly related to 5-FU, occurred in eight patients. No responses were seen in 14 evaluable patients with colon cancer, 11 of whom had had prior 5-FU. One patient with Hodgkin's disease had a partial response; one patient with diffuse histiocytic lymphoma had transient disease regression. Although allopurinol does modify the toxicity of 5-FU, permitting dose escalation, it does not increase the therapeutic index in colon cancer. Infusional 5-FU deserves further study in lymphoma.

[Cancer Treat Rep 66:1133–1137, 1982]

Chemotherapy for advanced colon cancer is generally ineffective. 5-FU is the most active agent, with a response rate of 15%–20% (1,2). Several different dose schedules have been utilized without clearly establishing an optimal schedule; however, iv administration appears to be superior to oral administration (3) and continuous iv infusion permits administration of higher doses than do single rapid injections (4). When administered by continuous infusion the maximum tolerated dose is $1.1\ g/m^2$/day, with mucositis being the dose-limiting toxicity (5). The response rate of 5-FU is related to drug-induced neutropenia (6), suggesting a steep dose-response curve (7).

To be cytotoxic, 5-FU must be converted to one of its two active nucleotide forms, FdUMP and FUTP (fig 1). FdUMP is a potent inhibitor of thymidylate synthetase and interferes with DNA synthesis (8). FUTP is incorporated into RNA and interferes with its stability and function (9). These nucleotides can be formed by either of two pathways: via the sequential action of uridine phosphorylase and uridine kinase or via the action of orotate phosphoribosyltransferase (OPRTase). The activation of 5-FU via thymidine phosphorylase is severely limited in man by a deficiency of deoxyriboside. Allopurinol can inhibit the conversion of 5-FU to its active metabolite by indirect inhibition of OPRTase (10). As shown in figure 1, the putative mechanism involves conversion of allopurinol to oxypurinol (11), which is phosphorylated to 1-oxypurinol-5′-monophosphate (12,13). The latter inhibits orotidylate decarboxylase (ODCase); this results in increases of intracellular levels of orotic acid, which effectively competes with 5-FU for OPRTase (11,14).

The route of 5-FU activation varies in different tissues. Schwartz and Handschumacher (15) have reported that allopurinol can reduce the toxicity of 5-FU to some cell lines in culture, reflecting the dependence of those cell lines on OPRTase for activation of 5-FU. Enhanced selectivity of 5-FU in combination with allopurinol might be expected in two circumstances: (*a*) when normal tissues depend

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[1] Received Aug 31, 1981; accepted Dec 16, 1981.

[2] Supported by Public Health Service grant CA-23100 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and a grant from the Clayton Foundation—California Divison. Dr. Howell is a Clayton Foundation Investigator.

[3] Department of Medicine and the Cancer Center, University of California, San Diego, La Jolla.

**Reprint requests to:* Joan F. Kroener, MD, Scripps Clinic and Research Foundation, 10666 N Torrey Pines Rd, La Jolla, CA 92037.

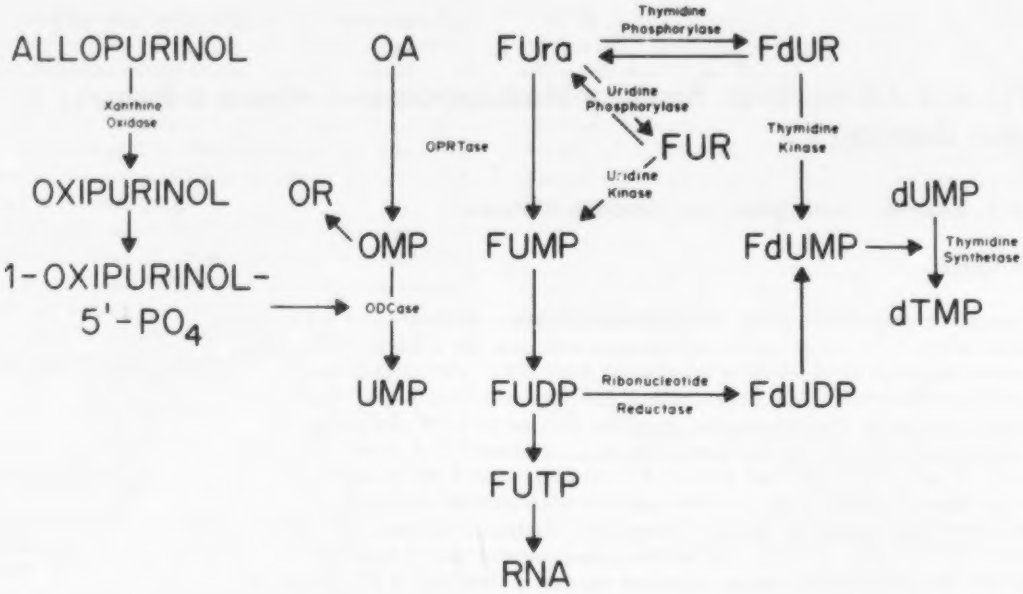

FIGURE 1.—Metabolic pathways of 5-FU and allopurinol. OA = orotic acid, OMP = orotidine monophosphate, OR = orotidine, UMP = uridine monophosphate, FUra = 5-FU, FUMP = 5-fluorouridine monophosphate, FUDP = 5-fluorouridine diphosphate, FUTP = 5-fluorouridine triphosphate, FdUR = 5-fluorodeoxyuridine, FdUMP = 5-fluorodeoxyuridine monophosphate, FdUDP = 5-fluorodeoxyuridine diphosphate, dUMP = deoxyuridine monophosphate, and dTMP = thymidine monophosphate.

primarily on the OPRTase pathway and malignant tissues depend on the uridine phosphorylase-uridine kinase pathway for activation of 5-FU; and (*b*) when the malignant tissue contains substantially higher levels of OPRTase so that an allopurinol concentration just sufficient to protect normal tissues would not completely inhibit the enzyme in the tumor. Under either of these conditions it may be possible to increase the tolerated dose of 5-FU and to improve the response rate.

Studies in murine tumor models have indicated that allopurinol can reduce toxicity and enhance the therapeutic ratio of 5-FU against some tumors but not others (16). Fox et al (17,18) conducted a phase I trial of 5-FU given by continuous infusion for 5 days with allopurinol at a dose of 300 mg orally every 8 hours starting 24 hours before and continuing throughout the period of 5-FU infusion. They reported that the 5-FU dose could be escalated by approximately a twofold factor and that a dose of 2 g/m^2/day of 5-FU produced objective responses in five of ten patients with colorectal carcinoma. The ability of allopurinol to protect marrow and gut against 5-FU toxicity has been confirmed at this institution (19,20). We now report our cumulative experience with the toxicity of the 5-FU/allopurinol program and phase II results in colon cancer.

MATERIALS AND METHODS

Patients

A total of 49 patients were entered in phase I and II studies. All patients had histologically proven cancer, gave informed consent, had failed or refused all standard therapy, and had recovered from any toxic effects associated with prior treatment. Eligibility requirements included wbc count > 3000/mm^3, platelet count > 100,000/mm^3 (except patients with leukemia or marrow replacement by tumor), serum creatinine < 1.5 mg/100 ml, life expectancy > 2 months, and ability to ingest oral medications. Phase I patients were also required to have bilirubin < 3 mg/100 ml, alkaline phosphatase increased less than twofold, and SGOT elevated less than threefold. Phase II patients were not excluded because of liver function abnormalities.

Study Design and Treatment Plan

5-FU was administered by continuous iv infusion for 5 days. In the phase I study, allopurinol at a dose of 300 mg was given orally every 8 hours beginning 2 hours prior to and ending 24 hours after 5-FU. In the phase II study, allopurinol was begun

24 hours prior to 5-FU and ended concurrently. No difference in the pattern of toxicity was observed with these two regimens. The starting dose of 5-FU was 1.5 g/m² day unless there was evidence of liver dysfunction (bilirubin > 3 mg/100 ml, alkaline phosphatase increased more than twofold, or SGOT elevated more than threefold), in which case phase II patients received an initial dose of 1 g/m²/day. In subsequent courses, the dose was escalated by 0.5–2 g/m²/day if there was no significant toxicity and no evidence of disease progression. Treatment was repeated every 4 weeks. Patients had blood cell counts and were examined for toxic effects weekly. Disease status was reassessed before each cycle. A partial response was defined as a decrease ≥ 50% in the product of the perpendicular diameters of measurable lesions lasting > 1 month.

RESULTS

The incidence of toxic effects associated with 81 courses of treatment in 49 patients entered in phase I and II studies is shown in table 1. The dose-limiting toxicity was mucositis, which was graded on a scale from 0 to IV: 0 = normal, grade I = oral pain without visible lesions, grade II = observable lesions with mild impairment of oral intake, grade III = moderate impairment of oral intake, and grade IV = no oral intake possible. Mucositis of grade II or greater occurred in 65% of courses at a dose of 1.5 g/m²/day of 5-FU and in 76% of courses at 2.0 g/m²/day. Mucositis appeared as early as Day 3, but usually on Day 6 or 7, and lasted an average of 1 week. No patient required hospitalization for iv fluids. Marrow toxicity was mild, with leukopenia (wbc count < 2000/mm³) occurring in 7% of evaluable courses and thrombocytopenia (platelet count < 100,000/mm³) occurring in 16%. One patient treated with 2 g/m²/day of 5-FU developed severe granulocytopenia (wbc count < 500/mm³) and died from sepsis on Day 14 of his first course of treatment. No clinically significant bleeding episodes occurred. Marrow suppression never caused a delay in treatment. A rash developed in 26% of evaluable courses. This was usually a maculopapular eruption on the trunk and was readily managed with symptomatic measures. In no case did skin rash prevent re-treatment. Nausea was not a significant problem.

Eight episodes of neurotoxicity, possibly 5-FU-related, occurred in 79 evaluable courses of treatment. Four episodes were encountered in the phase I study and consisted of a single focal motor seizure in one patient, a 10-minute episode of aphasia and hemianesthesia in the second, dysphagia and slurred speech in the third patient, who had progressive astrocytoma, and an acute syndrome of brainstem dysfunction followed by recurrent generalized seizures and death in the fourth. A common risk factor among these four patients was the use of phenothiazines. Their use was prohibited in the phase II study. There were four subsequent episodes of possible neurotoxicity. One patient had an episode of transient hemiparesis, with a negative workup for metastatic disease. A second patient had an episode of ataxia felt to be due to basilar artery insufficiency. A third patient had an episode of acute confusion with a negative neurologic evaluation; the episode resolved while treatment continued and did not recur with a second course of treatment. A fourth patient had an episode of aphasia followed by refractory hypotension and death thought possibly to be due to 5-FU cardiotoxicity.

A summary of the histologic diagnoses of patients treated in the phase I and II studies is presented in table 2. Of the 49 patients entered, 34 were evaluable for response because they received at least one course of therapy, survived at least 1 month, and had measurable disease. None of 14 evaluable patients with colon cancer had a response, yielding a probability of < 5% that the true response rate was > 20% (21). Eleven of the 14 patients had received prior treatment with 5-FU in low doses. The only responses seen were in patients with lymphoma. One heavily pretreated patient with Hodgkin's disease had near-complete resolution of bulky disease lasting for 4 months until he returned to

TABLE 1.—Incidence of toxicity

5-FU dose (g/m²)	No. of courses started	Incidence of toxicity/No. of evaluable courses						
		Mucositis			Leukopenia*	Thrombocytopenia†	Rash	Neurotoxicity
		I	II	III				
1	6	1/6	1/6	0/6	0/4	0/4	0/6	0/6
1.5	51	3/43	19/43	9/43	2/34	4/34	8/46	7/50
2	24	2/21	4/21	12/21	2/18	5/18	11/22	1/23

*Wbc count < 2000/mm³.
†Platelet count < 100,000/mm³.

TABLE 2.—Clinical responses to 5-FU and allopurinol therapy

Tumor type	Total No. of patients	No. of evaluable patients	Response*
Colorectal	18	14	NR
Lung			
Non-oat cell	7	4	NR
Oat cell	3	0	—
Lymphoma	4	4	1 PR (4 mos)
Adenocarcinoma (unknown primary)	3	3	NR
Melanoma	3	3	NR
Breast	2	1	NR
Miscellaneous	9	5	NR

*PR = partial response, NR = no response.

Mexico and was lost to followup. A second patient with diffuse histiocytic lymphoma had a > 50% decrease in the size of his abdominal mass. The duration of the response, however, was only 2 weeks; thus, he is not scored as a responder. The only other patient with objective improvement was a heavily pretreated patient with acute lymphocytic leukemia who had transient improvement in her pancytopenia and decrease in the circulatory blast cell count following 5-FU and allopurinol therapy.

DISCUSSION

Allopurinol does increase the tolerated dose of 5-FU in man. In previous reports the maximum tolerated dose of 5-FU given as a 5-day constant infusion was 1.1 g/m^2/day (5). With allopurinol, the dose could be escalated to 1.5 g/m^2/day in all patients and to 2 g/m^2/day in some patients. Toxicity associated with this treatment program was predominantly mucositis, with minimal marrow suppression. Eight patients had possible neurotoxicity and one of them died. No cases of acute cerebellar toxicity occurred, as previously described with bolus 5-FU (22). Neurologic evaluation failed to reveal anything other than 5-FU exposure as the cause of the dysfunction in five patients; the other three patients were found to have disease processes that could reasonably explain their symptoms. Whether the 5-FU was responsible for the symptoms in the five patients without other etiologies cannot be determined. Any patient receiving 5-FU and allopurinol treatment should be monitored carefully for neurotoxicity.

Despite the theoretic potential for improving responses to 5-FU by allowing administration of higher doses and possibly by selectively protecting normal tissues, there was no suggestion of an improvement in the response rate in colon cancer. None of 14 patients with advanced colon cancer responded. One contributing factor may have been that most of these patients had received prior 5-FU therapy and may have developed tumor resistance to it. The possibility that the allopurinol modulated the toxicity of 5-FU with equal effectiveness in the tumor and in the normal tissues must also be considered. We could not confirm the 50% response rate reported by Fox et al (17) in gastrointestinal adenocarcinomas. It should be noted, however, that those responses were seen in patients treated with 2.25 g/m^2/day of 5-FU, a dose we were unable to attain without unacceptable toxicity.

The one partial response and one transient improvement in two patients with lymphoma suggest that infusional 5-FU may have activity in lymphoma. Since no data on 5-FU infusion without allopurinol are available in lymphoma, we cannot conclude that the addition of allopurinol played a role in these responses. However, this treatment program merits further study in lymphoma patients refractory to other therapy.

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Safety Assessment of a New Anticancer Compound, Mitoxantrone, in Beagle Dogs: Comparison With Doxorubicin. I. Clinical Observations[1,2]

B. M. Henderson,[3] W. J. Dougherty,[3,*] V. C. James,[3] L. P. Tilley,[4] and J. F. Noble[3]

SUMMARY

Twenty-four adult beagle dogs were divided into four groups of three males and three females and received iv infusions of doxorubicin (36.05 mg/m^2), mitoxantrone (2.58 or 5.15 mg/m^2), or the vehicle (0.9% normal saline). All animals were given a single dose once every 3 weeks. The duration of the study was 30 weeks. Animals were observed for toxicologic and cardiotoxic signs. The methods used to evaluate the cardiotoxic potential of both mitoxantrone and doxorubicin were sequential endomyocardial biopsies, ECGs, blood pressure, and serum levels of the cardiospecific isoenzyme CPK-MB (MB band of CPK). Animals given mitoxantrone had signs of gastrointestinal toxicity and fluctuating decreases in wbc counts. Animals given doxorubicin had signs of gastrointestinal toxicity and cardiotoxicity, as well as alopecia, fluctuating decreases in wbc counts, and diffuse erythema. All three male animals given doxorubicin died during the study from apparent congestive heart failure. All dogs treated with doxorubicin had positive CPK isoenzyme elevation, ECG changes, or progressive cardiomyopathy prior to administration of the last dose. None of these signs was observed in dogs treated with mitoxantrone. One male dog given mitoxantrone died during the course of the study.

[Cancer Treat Rep 66:1139–1143, 1982]

The clinical usefulness of doxorubicin has stimulated research to discover new agents with similar mechanisms of action without the dose-limiting cardiotoxicity of anthracyclines. The mechanism of action of mitoxantrone is similar to that of doxorubicin, ie, it intercalates with DNA (1). Because of this, it was thought useful to compare the potential cardiotoxicity of these two agents.

Mitoxantrone has been found by Wallace et al (1) and Murdock et al (2) to be an effective chemotherapeutic agent against several experimental tumor systems in mice. Studies in mice have demonstrated that treatment with mitoxantrone resulted in increased lifespan when the compound was tested against P388 and L1210 leukemias as well as the solid neoplasms B16 melanoma and colon tumor 26. Cheng et al (3) have also described the antitumor capability of the parent of mitoxantrone. All studies showed that mitoxantrone is at least as effective as doxorubicin, and in some instances more effective than doxorubicin, in the tumor systems previously mentioned.

The cumulative cardiotoxicity that results from some of the anthracycline antibiotics is well-documented, especially for doxorubicin (4–7). Several models have been used to study this effect, including the rat (8–10), rabbit (9,10), mouse (9,10), mon-

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[1] Received Oct 28, 1980; revised Dec 1, 1981; accepted Dec 17, 1981.

[2] Portions of these data were presented at the Nineteenth Annual Meeting of the Society of Toxicology, Washington, DC, Mar 11, 1980.

[3] Toxicology Research, Medical Research Division, American Cyanamid Co, Pearl River, NY.

[4] Cardiovascular Department, Animal Medical Center, New York, NY.

**Reprint requests to*: Dr. W. J. Dougherty, Toxicology Evaluation Department, Medical Research Division, American Cyanamid Co, Pearl River, NY 10965.

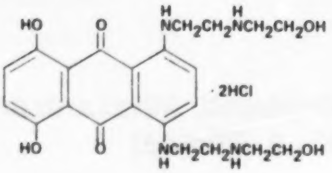

FIGURE 1.—Structural formula for mitoxantrone dihydrochloride.

key (11,12), pig (13), and dog (14–16). Methods (17–20) have been developed that attempt to predict the time and dose at which cardiotoxicity develops, thereby indicating the time of discontinuance of drug administration.

As part of the preclinical evaluation of mitoxantrone, several safety studies have been conducted. The iv LD50 in rats is 4.8 mg/kg for males and 5.2 mg/kg for females (unpublished data). The iv LD50 in mice is 11.3 mg/kg for males and 9.7 mg/kg for females (unpublished data). The results of several studies in beagle dogs have indicated that 10.3 mg/m^2 is the minimum single lethal dose of mitoxantrone given iv (unpublished data). Several authors (14,15) have shown that the dog is a useful model for assessing the cardiotoxic potential of doxorubicin and other antitumor agents. The assessment of toxicity and of cardiotoxic potential of mitoxantrone compared with doxorubicin is the subject of the present report. Postmortem data are presented in a separate report (21).

MATERIALS AND METHODS

Mitoxantrone, 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride, has the structure shown in figure 1.

Twenty-four adult Marshall Research beagle dogs, approximately 12–14 months of age at the start of dosing, were divided into four groups of three males and three females each. The experimental design is presented in table 1. The dosing regimen consisted of a single iv administration once every 3 weeks, repeated nine or ten times depending on the survival of the animals. Based on preliminary studies (unpublished data), the high dose (5.15 mg/m^2) of mitoxantrone is approximately one half the single lethal dose in dogs, and 36.05 mg/m^2 of doxorubicin is one half the lethal dose in dogs (22). These doses were found to be equitoxic based on the comparable reductions in peripheral wbc counts induced by both compounds. In addition, they were selected because they were considered to be the maximum tolerated dose in dogs. The dosing regimen is similar to that used for doxorubicin clinically and is the one anticipated to be used clinically for mitoxantrone. Doses of mitoxantrone are expressed as the base, and doses of doxorubicin as the hydrochloride.

Solutions of mitoxantrone and doxorubicin were prepared fresh on the day of dosing in 0.9% sterile saline. Iv infusions of mitoxantrone, doxorubicin, or the vehicle were given to unanesthetized dogs through a 22-gauge needle set into either the right or left saphenous vein; a constant rate of 0.4 ml/kg/minute was maintained for 5 minutes.

ECGs were taken on the day prior to each dose and one or two times between doses using a Hewlett-Packard 1507A Vectron Programmer, 1508 Lead Network, and mobile chart drive 1064A. The leads recorded were I, II, III, AVR, AVL, AVF, and CVX. A 22-gauge stainless steel needle connected to the lead wires was placed sc into each foreleg and hindleg and over the xyphoid process of the sternum. Each lead was recorded for 10 seconds. Arterial blood pressure was taken on the same schedule as the ECG using a Statham Physiological Pressure Transducer, Model P23GB, connected to an 8805C Hewlett-Packard pressure amplifier. All measurements were recorded with the dogs unanesthetized, restrained, and in dorsal recumbency.

TABLE 1.—Experimental design and mortality

Compound	No. of dogs		Dose*		Mortality		Survival time (days)	
	Male	Female	Mg/kg	Mg/m^2†	Males	Females	Mean	Range
0.9% NaCl	3	3	0	0	0	0‡	215.0	214–216
Mitoxantrone	3	3	0.125	2.58	0	0	215.0	214–216
Mitoxantrone	3	3	0.25	5.15	1	0	197.8	116–215
Doxorubicin	3	3	1.75	36.05	3	3§	170.0	154–174

*Administered once every 3 wks.

†Calculated using a km factor (conversion of mg/kg to mg/m^2) of 20.6.

‡1 dog killed after 25 wks is not included.

§3 dogs killed after 25 wks are included.

Evaluation of the ECG included analysis of heart rate, heart rhythm, amplitude, and duration of P-QRS-T complex, P-R interval, S-T segment changes, and mean electrical axis in the frontal plane.

Hematologic determinations were performed on the day prior to each dose and 7 days after each dose. Serum parameters, including enzymes, were measured on the day after each dose.

Sequential endomyocardial biopsies of the right ventricular septum were performed about 2 weeks after the fourth (or fifth), seventh, and ninth doses using a modified technique described for patients (23) and dogs (unpublished data). Prior to the biopsy procedure, the dogs were given atropine and anesthetized with a mixture of ketamine and diazepam in a 2:1 ratio.

RESULTS

Mortality is summarized in table 1. Three male dogs given doxorubicin were found dead during the study after 22, 24, and 25 weeks (cumulative doses of 288.4 or 325.4 mg/m^2). These animals died of apparent congestive heart failure. The signs of heart failure included fluid sounds in the chest cavity on auscultation; edematous swelling of the head, trunk, and limbs; weakness (inactivity); and increased heart rates. Other signs in these animals included an erythematous reaction in the region of the head, neck, and trunk; diarrhea that progressed to bloody diarrhea; alopecia; emesis; increased salivation; and soft stools. Female animals receiving doxorubicin (cumulative dose of 325.45 mg/m^2) were killed after 25 weeks; they exhibited all the signs described above, except fluid sounds in the chest.

Signs of toxic effects observed in animals that received the high dose of mitoxantrone (5.15 mg/m^2) included soft stools, diarrhea or bloody diarrhea, emesis, increased salivation, cutaneous sores, swelling of the extremities, and inactivity. One male in this group died of pneumonia during the 17th week, 2 weeks after an endomyocardial biopsy was performed. This animal had received a cumulative dose of 30.9 mg/m^2 of mitoxantrone.

Dogs given the low dose of mitoxantrone (2.58 mg/m^2) had soft stools and an occasional emetic episode. Also noted in one animal were diarrhea and edematous swelling of the extremities. Animals given the vehicle (0.9% sterile saline) had soft stools, and one animal from this group had diarrhea and edematous swelling of the extremities. A single emetic episode was also observed in one animal given the vehicle.

There were dose-related reductions in the mean hematocrit, hemoglobin concentration, and wbc counts in both mitoxantrone- and doxorubicin-treated dogs. The reductions in these parameters were observed 6 days after each administration and in some cases were statistically significant as compared to controls. The reductions in these values tended to return to predose values prior to the next administration of either mitoxantrone or doxorubicin. The reticulocyte count of all treated animals was reduced after the administration of each dose but tended to return to normal between doses.

Reductions were observed in the peripheral wbc counts of all treated dogs; these reductions were attributed to decreases in peripheral neutrophils and lymphocytes. Reductions were noted after administration of the first dose but were not statistically significant until after administration of the third dose. The pattern of reduction in wbc counts after the administration of each dose, followed by an increase toward normal between doses, is illustrated in figures 2 and 3. The magnitude of the change in wbc counts was similar for both mitoxantrone and doxorubicin. Based on this, the doses of mitoxantrone and doxorubicin were considered to be equitoxic.

A significant ($P < 0.05$) elevation in the cardiospecific isoenzyme CPK-MB was observed after 24 weeks (1 day after administration of the ninth dose) in four of six dogs treated with doxorubicin. One animal treated with doxorubicin had a transient elevation in LDH-4 isoenzyme 1 day after administration of the sixth dose. There were no changes in any of the other serum chemistry parameters measured that could be related to either mitoxantrone or doxorubicin treatment.

Changes in the ECGs of dogs treated with doxorubicin were first detected in two animals after 16 weeks, 1 week after administration of the sixth dose. At that time, ventricular and atrial premature complexes were observed. These two animals had received cumulative doses of 228.3 mg/m^2 of doxorubicin. After 21 weeks, five of six animals given doxorubicin had ECG changes, including QRS voltage reductions ($\geq 40\%$), inverted T waves, atrial ectopic complexes, sinus tachycardia, and widening of the QRS complex. All animals of this group had ECG changes after the eighth dose (cumulative dose of 288.4 mg/m^2), including changes previously noted as well as left intraventricular conduction defects and a widening of the P wave (one dog). These changes persisted until the death of the animals. QRS notching was seen in one female prior to being killed (cumulative dose of 344.45 mg/m^2). There were no ECG changes in any of the animals given mitoxantrone.

Reductions in mean arterial blood pressure were observed in three of six animals given doxorubicin.

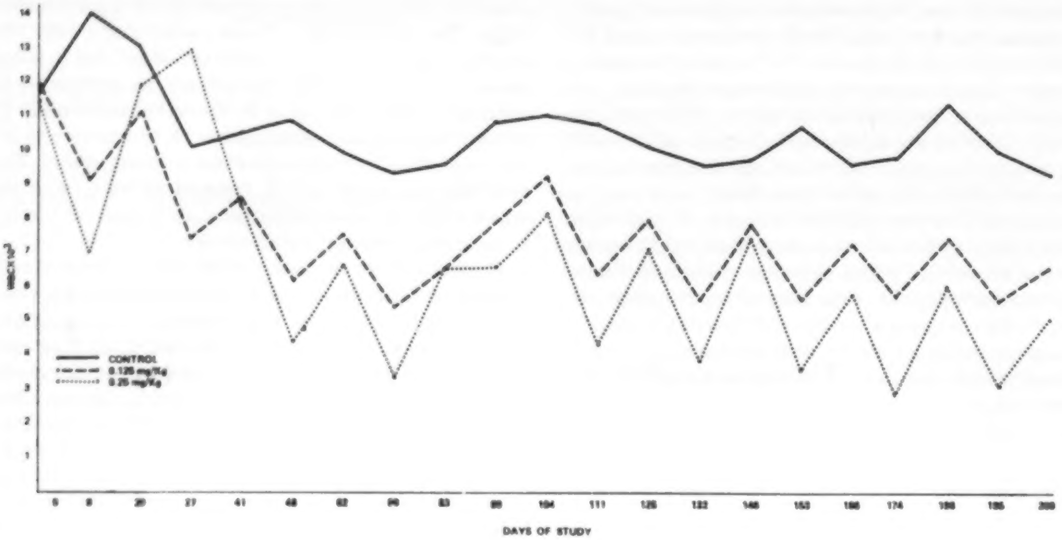

FIGURE 2.—Fluctuations in peripheral wbc counts related to time of administration of each dose of mitoxantrone, showing pattern of reduction of wbcs after administration of each dose and increases between doses.

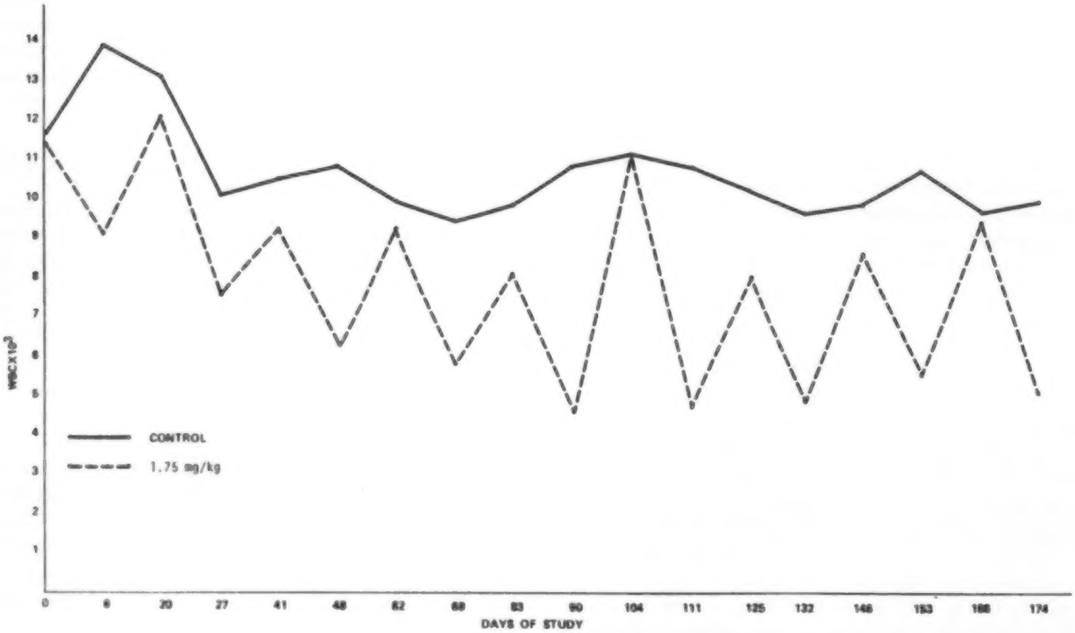

FIGURE 3.—Fluctuations in peripheral wbc counts related to time of administration of doxorubicin, showing pattern of reduction of wbcs after administration of each dose and increases between doses.

A reduction of 41%–46% was observed in these animals by the time of their death. Reductions were seen initially during the 21st week (cumulative dose of 252.35 mg/m^2), but three animals had transient reductions in blood pressure by the tenth week (cumulative dose of 144.2 mg/m^2). There were no changes in blood pressure of any of the animals given mitoxantrone.

DISCUSSION

The results of this study indicate that mitoxantrone administered iv once every 3 weeks to dogs, at doses of 2.58 and 5.15 mg/m^2 repeated ten times, was not cardiotoxic. In dogs treated with doxorubicin, changes indicative of cardiotoxicity occurred in the ECGs at a cumulative dose of 228.3 mg/m^2. This agrees with the observation of morphologic changes indicative of cardiotoxicity (21). However, the morphologic changes were initially observed at a cumulative dose of 144 mg/m^2 of doxorubicin prior to any clinical signs. There was no clinical evidence of cardiotoxicity in mitoxantrone-treated dogs. This agrees with the absence of morphologic changes indicative of progressive cardiomyopathy (21).

During this study, there was clinical evidence of both gastrointestinal toxicity and myelosuppression in dogs treated with either mitoxantrone or doxorubicin. The myelosuppression, as indicated by changes in peripheral wbc counts, was similar in dogs treated with the highest dose of mitoxantrone and with doxorubicin, suggesting that the two doses were equitoxic.

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Safety Assessment of a New Anticancer Compound, Mitoxantrone, in Beagle Dogs: Comparison With Doxorubicin. II. Histologic and Ultrastructural Pathology[1,2]

B. M. Sparano,* G. Gordon, C. Hall, M. J. Iatropoulos, and J. F. Noble[3]

SUMMARY

Beagle dogs received either doxorubicin hydrochloride (1.75 mg/kg) or mitoxantrone (0.125 or 0.25 mg/kg) iv once every 3 weeks. These doses were equivalent to 36.05 mg/m² of doxorubicin and 2.58 or 5.15 mg/m² of mitoxantrone. Sequential endomyocardial biopsies were performed approximately 2 weeks after the fourth (or fifth), seventh, and ninth doses in order to monitor histopathologic and ultrastructural changes during the study. Myocardial lesions that progressed with time and dose were observed in heart samples from dogs that received doxorubicin, but not in dogs that received mitoxantrone. The myocardial lesions induced by doxorubicin were observed with cumulative doses as low as 144 mg/m². Myocardial changes, which did not progress with time and cumulative dose, were observed in dogs that received either dose of mitoxantrone. The earliest observable evidence of doxorubicin-associated cardiotoxicity was seen morphologically in biopsy material before clinical signs of cardiotoxicity. No evidence of cardiotoxicity, either morphologic or clinical, was seen in dogs treated with the maximum tolerated dose of mitoxantrone during the course of treatment. The dog appears to be a suitable model for studying the chronic cardiotoxic effects of anthracyclines and for monitoring effects of compounds such as mitoxantrone, which show a spectrum of activity and mechanism of action similar to that of anthracycline compounds.

[Cancer Treat Rep 66:1145–1158, 1982]

The clinical usefulness of doxorubicin has stimulated research to discover new agents with similar mechanisms of action that lack the dose-limiting cardiotoxicity of anthracyclines. Anticancer activity against experimental tumors in mice comparable to that of doxorubicin has been reported (1) for the anthracenedione derivative mitoxantrone hydrochloride, or 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione dihydrochloride. The mechanism of action of mitoxantrone is similar to that of doxorubicin, ie, it intercalates with DNA (1). Consequently, this study was undertaken to assess the acute and chronic cardiotoxic potential of mitoxantrone and to compare it with the known cardiotoxic effects of doxorubicin.

It has been shown (2) that anthracycline cardiomyopathy associated with chronic administration can be monitored in patients by sequential endomyocardial biopsies and that the progressive changes observed in the myocardium are predictive of heart failure. Recent studies have shown that doxorubicin produces cardiomyopathy in beagle dogs (3,4) similar to that observed in man during long-term administration of anthracyclines. The changes reported in dogs and other laboratory animals were

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[1] Received Oct 28, 1980; revised Dec 1, 1981; accepted Dec 17, 1981.

[2] Portions of these data were presented at the Nineteenth Annual Meeting of the Society of Toxicology, Washington, DC, March 11, 1980.

[3] Toxicology Research, Medical Research Division, American Cyanamid Co, Pearl River, NY.

**Reprint requests to*: B. M. Sparano, PhD, Experimental Pathology Department, Toxicology Research, Medical Research Division, American Cyanamid Co, Pearl River, NY 10965.

TABLE 1.—Mortality

Compound	Dose*		No. of dogs/ group	Mortality	Survival time (days)	
	Mg/kg	Mg/m^2			Mean	Range
0.9% NaCl	0	0	6	0†	214.8	214–216
Mitoxantrone	0.125	2.58	6	0	215.0	214–216
Mitoxantrone	0.25	5.15	6	1	197.8	116–215
Doxorubicin	1.75	36.05	6	6‡	170.0	154–174

*Administered once every 21 days.

†1 dog killed on Day 175 is not included.

‡3 dogs killed on Day 174 are included.

found at postmortem examination after extended periods of dosing and recovery.

By using sequential endomyocardial biopsies, we attempted to detect myocardial changes at an early stage in beagle dogs after administration of mitoxantrone or doxorubicin during a 30-week dosing schedule. The results of clinical observations in dogs treated with mitoxantrone and doxorubicin are reported separately (5). The results of histopathologic and electron microscopic observations are the subject of the present report.

MATERIALS AND METHODS

Twenty-four beagle dogs were randomly distributed into four groups of three males and three females each. Dogs that received normal saline iv were controls. Drug-treated dogs received either 0.125 or 0.25 mg/kg of mitoxantrone hydrochloride (doses expressed as parent) or 1.75 mg/kg of doxorubicin hydrochloride (1.64 mg/kg of doxorubicin). These doses were equivalent to 2.58 or 5.15 mg/m^2 of mitoxantrone and 36.05 mg/m^2 of doxorubicin. The dosing regimen consisted of a single iv administration once every 3 weeks repeated nine or ten times, depending on the survival of the animals.

Endomyocardial biopsies (5) were performed approximately 2 weeks after administration of the fourth (or fifth), seventh, and ninth doses. Surviving animals were killed for postmortem examination approximately 3 weeks after administration of the tenth dose, and heart samples were taken from the right ventricular septum immediately adjacent to the biopsy site.

Biopsy samples and heart samples taken at the postmortem examination were fixed in 2.5% glutaraldehyde in Sørensen's phosphate buffer (pH 7.4), washed in buffer alone, postfixed in 1.0% osmium tetroxide in phosphate buffer, dehydrated with ethanol, and embedded in Epon 812. Sections cut at 1 μ were stained with toluidine blue O and graded according to the system described by Billingham et al (2) using the light microscope. Thin sections (70–80 nm) mounted on uncoated grids were stained with uranyl acetate and Reynolds' lead citrate (6) for ultrastructural examination in a Philips 200 electron microscope at an accelerating voltage of 60 kV.

Samples of all organs were preserved in 10% phosphate-buffered Formalin, routinely processed, embedded in paraffin, and stained with hematoxylin and eosin for light microscopic examination.

RESULTS

Mortality is summarized in table 1. One dog that received 0.25 mg/kg of mitoxantrone died after 17 weeks (cumulative dose of 30.9 mg/m^2). The death of this animal was due to pulmonary infarction with abscess formation and necrotizing pleuritis. The three male dogs that received doxorubicin died after 22 or 24 weeks (cumulative doses of 288.4 and 324.45 mg/m^2). The three female dogs that received doxorubicin were killed after 25 weeks along with one saline-treated female in order to obtain unautolyzed heart samples for light and electron microscopic examination. Surviving dogs were killed for postmortem examination during the 30th week of the study.

Histopathology

Cardiotoxicity was observed only in dogs treated with doxorubicin. The changes in the myocardium are summarized in table 2, which shows that the cardiac lesions progressed with time and dose. The earliest gradable lesion consisted of coalesced vacuoles in occasional myocytes observed in plastic-embedded 1-μ sections stained with toluidine blue. The more severe manifestation of this lesion consisted of groups of cells with vacuolar degeneration and myocytolysis. Each of the three grades of myocytic change is illustrated in figure 1. No gradable lesions were observed in heart samples from dogs treated with mitoxantrone.

TABLE 2.—Progressive cardiomyopathy in dogs treated with doxorubicin

	Cumulative dose (mg/m²)	Grade*		Incidence†
		Mean	Range	
First biopsy	144.2	1.3	1–2	6/6
Second biopsy	252.35	2.3	2–3	6/6
Postmortem	324.45	3.0	3	3/3

*Based on light microscopic examination of 1-μ plastic-embedded sections stained with toluidine blue. 0 = normal; 1 = occasional cells with slight change; 2 = groups of cells with moderate change; 3 = numerous cells with severe change.

†No. of dogs with cardiomyopathy/No. examined.

Other histopathologic changes were testicular degeneration with aspermatogenesis in all drug-treated dogs. Atrophy of hair follicles, bone marrow hypocellularity, and lymphoid atrophy occurred only in dogs treated with doxorubicin. Also, hypocellularity of bone marrow and lymphoid depletion in lymph nodes and tonsils were observed in the dog treated with mitoxantrone that was found dead.

Ultrastructural Pathology

The myocardial lesions observed with the light microscope in dogs treated with doxorubicin were characterized ultrastructurally by dilatation of the sarcoplasmic reticulum with the formation of aggregates of individual vacuoles derived from the sarcoplasmic reticulum (fig 2). In some cases, coalesced larger vacuoles replaced the normal contractile elements of the myocyte (fig 3). A second lesion, not observed with the light microscope, consisted of disorientation and loss of myofilaments in individual myocytes (fig 4).

The ultrastructural appearance of postmortem samples of myocardium from some dogs treated with mitoxantrone (fig 5) was identical to that of myocardium from saline-treated dogs (fig 6). However, slight dilatation of the sarcoplasmic reticulum was observed in postmortem samples from one of the two dogs examined that received 0.125 mg/kg of mitoxantrone and from two of the four dogs examined that received 0.25 mg/kg of mitoxantrone. This change was more severe in biopsy samples (fig 7) than in postmortem samples (fig 8) from the same dogs. Dilated sarcoplasmic reticulum was observed to a lesser extent in biopsy samples from two saline-treated dogs (fig 9), although it was not present in postmortem samples from the same dogs.

DISCUSSION

The results of the present study indicate that the dog develops a progressive and chronic cardiomyopathy with administration of 36 mg/m² of doxorubicin once every 3 weeks. The progression of myocardial changes, first observed after a cumulative dose of 144 mg/m², is similar to that reported for man (2), in whom the limiting dose appears to be 400–500 mg/m². Cardiomyopathy after doxorubicin administration has also been reported in the rabbit (7), rat (8), mouse (9), monkey (3,10), and pig (11). However, our data support the conclusions of recent studies (4,12) that the dog is a highly reproducible and useful model for studies of anthracycline cardiotoxicity in man. The present study has also shown that progressive anthracycline cardiomyopathy can be monitored by morphologic changes in sequential endomyocardial biopsies during chronic administration in a manner similar to that used in patients treated with doxorubicin (2). These morphologic changes in dogs occur earlier than changes in ECGs and serum enzymes (5).

The two ultrastructural lesions seen in dogs treated with doxorubicin have been reported to occur in patients undergoing doxorubicin therapy (2); however, loss of myofibrils is seen more commonly in patients than in dogs (4). The present findings of dilated sarcoplasmic reticulum and of myofibrillar loss seen in dogs after a cumulative dose of 252 mg/m² agree with a recent report (4) in which both lesions were observed in dogs after a cumulative dose of 340–400 mg/m².

A progressive cardiomyopathy was not observed with the administration of mitoxantrone. Although dilatation of the sarcoplasmic reticulum was observed in the final biopsy samples, it was not seen to the same extent or was absent in postmortem samples from the same dogs. It was a transient effect that was not dose-related; therefore, it was not considered to be indicative of a dose-limiting cardiomyopathy in dogs treated with mitoxantrone. Since dilatation of the sarcoplasmic reticulum to a lesser extent was also seen in biopsy samples from two saline-treated dogs, it is possible that mechanical damage from the biopsy procedure was responsible, in part, for this effect. Whether or not dose-

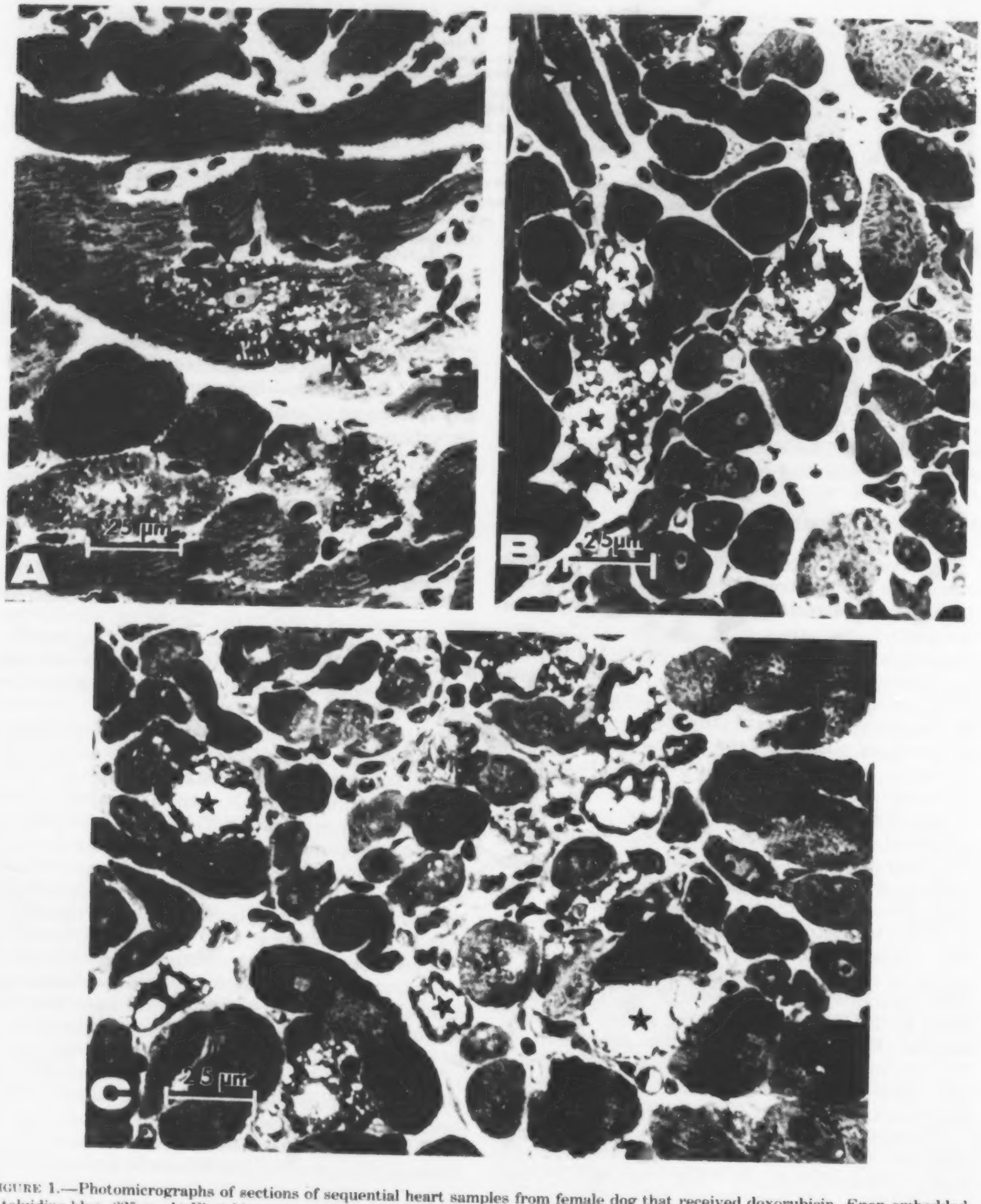

FIGURE 1.—Photomicrographs of sections of sequential heart samples from female dog that received doxorubicin. Epon-embedded, toluidine blue, 625 ×. A) First biopsy (cumulative dose of 144 mg/m^2), illustrating grade 1 cardiomyopathy; a few myocytes contain numerous small vacuoles (arrows). B) Second biopsy (cumulative dose of 252 mg/m^2), illustrating grade 2 cardiomyopathy; groups of cells contain small vacuoles (arrows) and large vacuoles (stars). C) Postmortem sample (cumulative dose of 325 mg/m^2), illustrating grade 3 cardiomyopathy; numerous myocytes contain large vacuoles (stars) that have replaced the normal contractile elements.

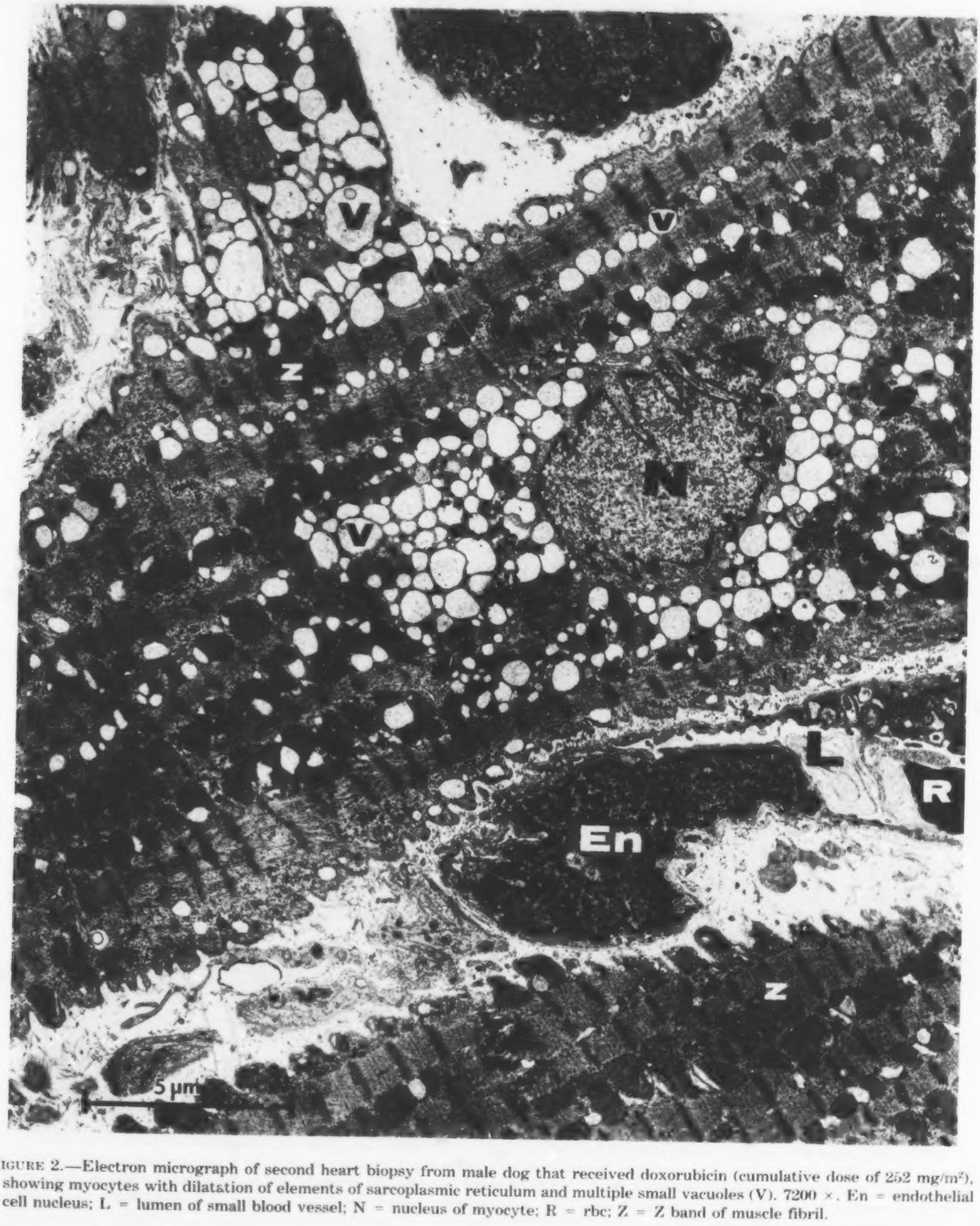

FIGURE 2.—Electron micrograph of second heart biopsy from male dog that received doxorubicin (cumulative dose of 252 mg/m^2), showing myocytes with dilatation of elements of sarcoplasmic reticulum and multiple small vacuoles (V). 7200 ×. En = endothelial cell nucleus; L = lumen of small blood vessel; N = nucleus of myocyte; R = rbc; Z = Z band of muscle fibril.

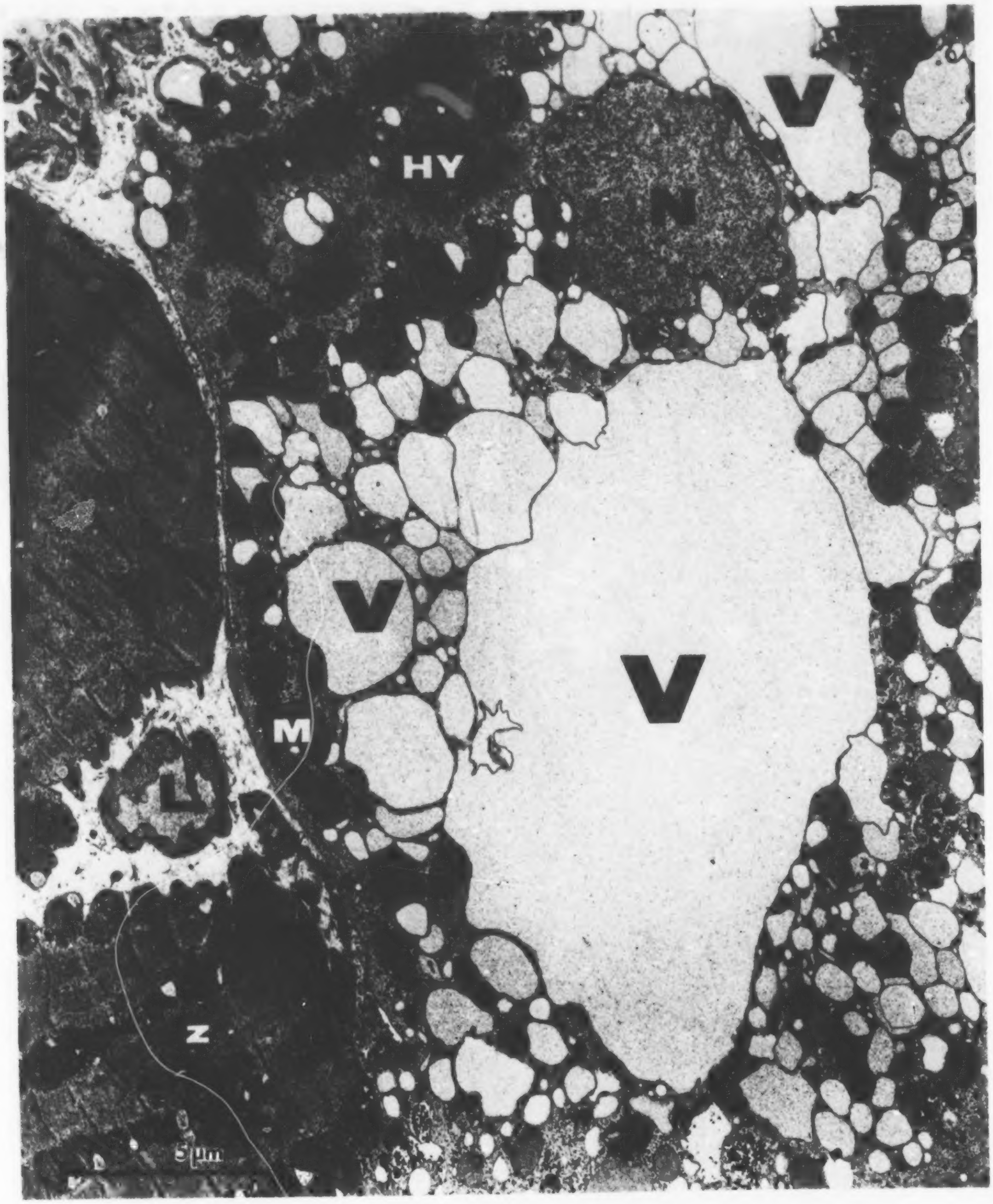

FIGURE 3.—Electron micrograph of second heart biopsy from same male dog as sample shown in fig 2, showing portions of 3 myocytes, 2 of which appear to be normal. Third myocyte is filled with large vacuoles (V) derived from sarcoplasmic reticulum. 7200 ×. HY = hypercontracted myofibril; L = capillary lumen; M = mitochondrion; N = nucleus of myocyte; Z = Z band of muscle fibril.

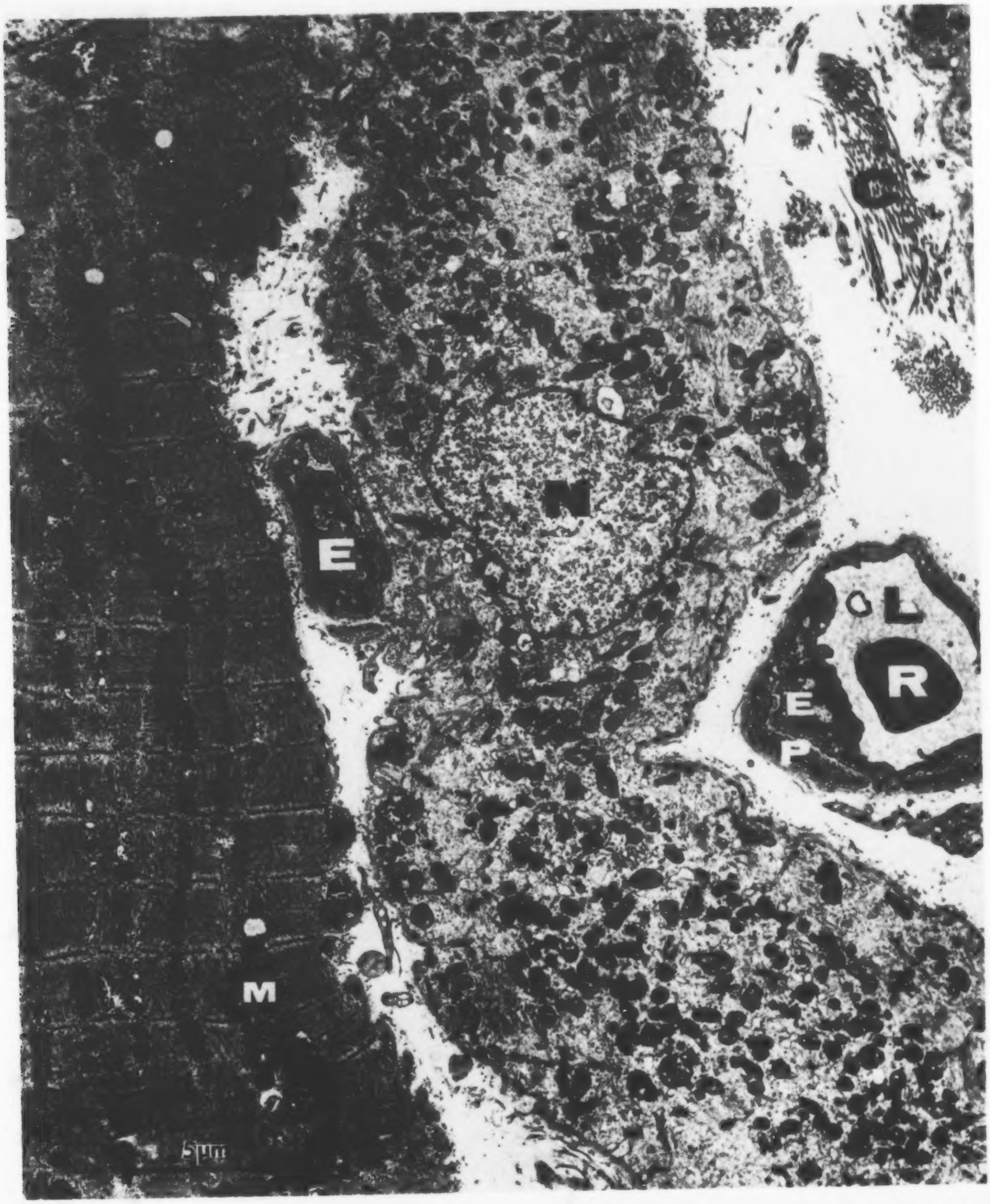

FIGURE 4.—Electron micrograph of heart sample taken postmortem from female dog that received doxorubicin (cumulative dose of 325 mg/m^2). Myocyte on the right exhibits disorientation and loss of myofilaments, while myocyte on the left appears to be normal. 7200 ×. C = collagen; E = endothelial cell nucleus; L = lumen of small blood vessel; M = mitochondrion; N = nucleus of myocyte; P = pericyte; R = rbc; Z = Z band of muscle fibril.

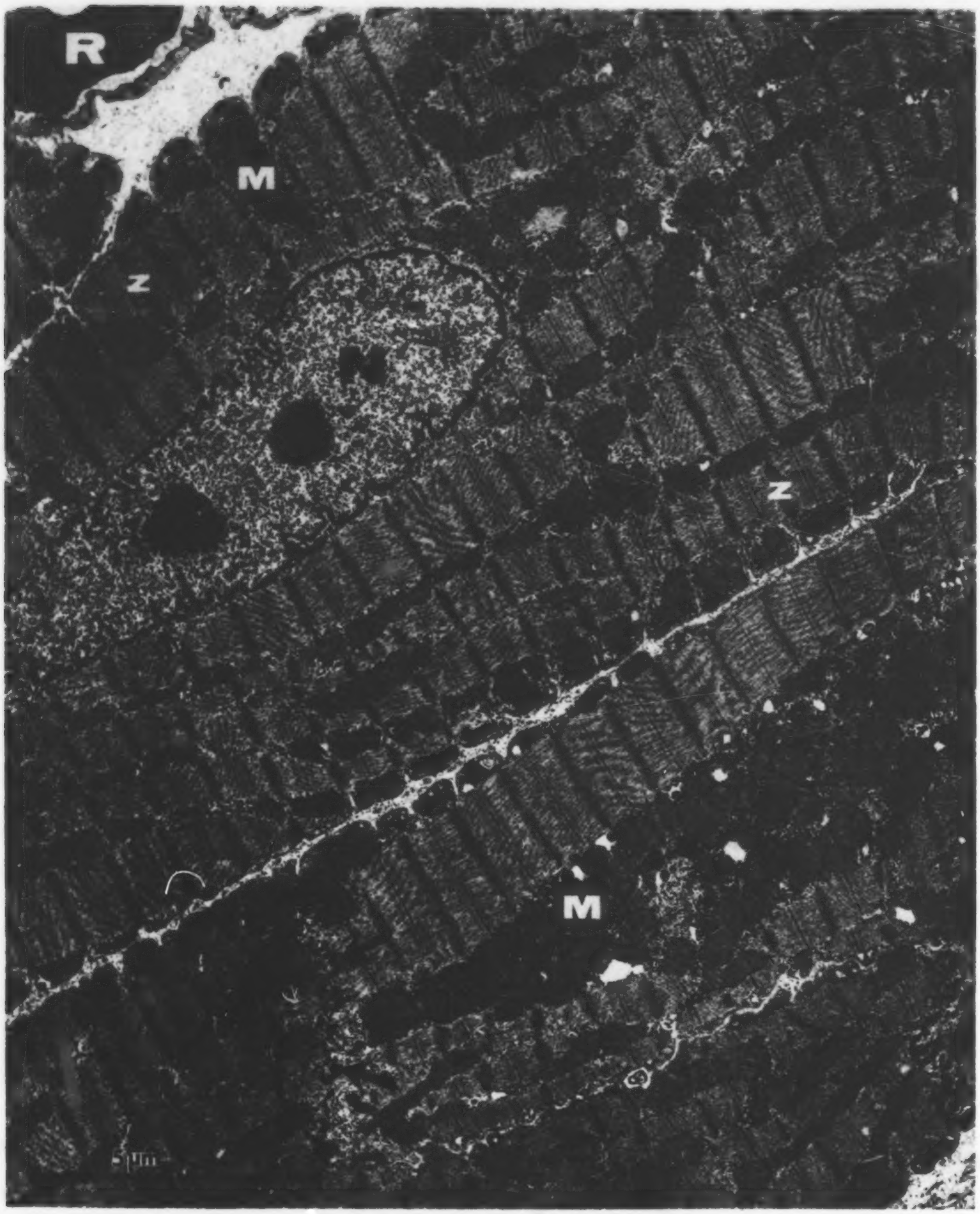

FIGURE 5.—Electron micrograph of heart sample taken postmortem from female dog that received 0.25 mg/kg of mitoxantrone (cumulative dose of 52 mg/m^2), showing portions of normal myocytes. Arrows indicate intercalated disc. 9300 ×. M = mitochondrion; N = nucleus of myocyte; R = rbc in lumen of small blood vessel; Z = Z band of myofibril.

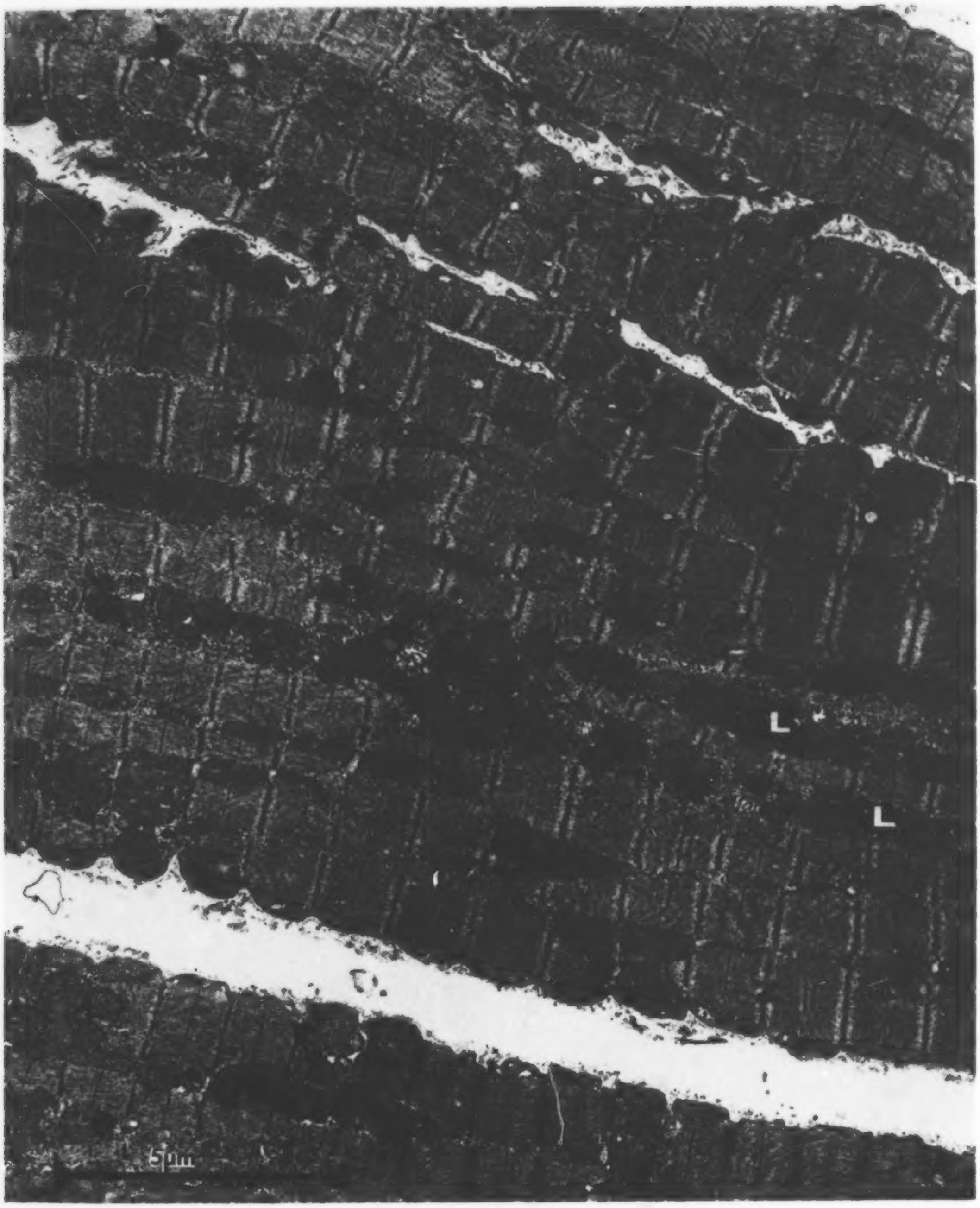

FIGURE 6.—Electron micrograph of heart sample taken postmortem from saline-treated male dog, showing portions of normal myocytes. Arrow indicates intercalated disc. 9300 ×. L = lipofuscin deposit; M = mitochondrion; Z = Z band of myofibril.

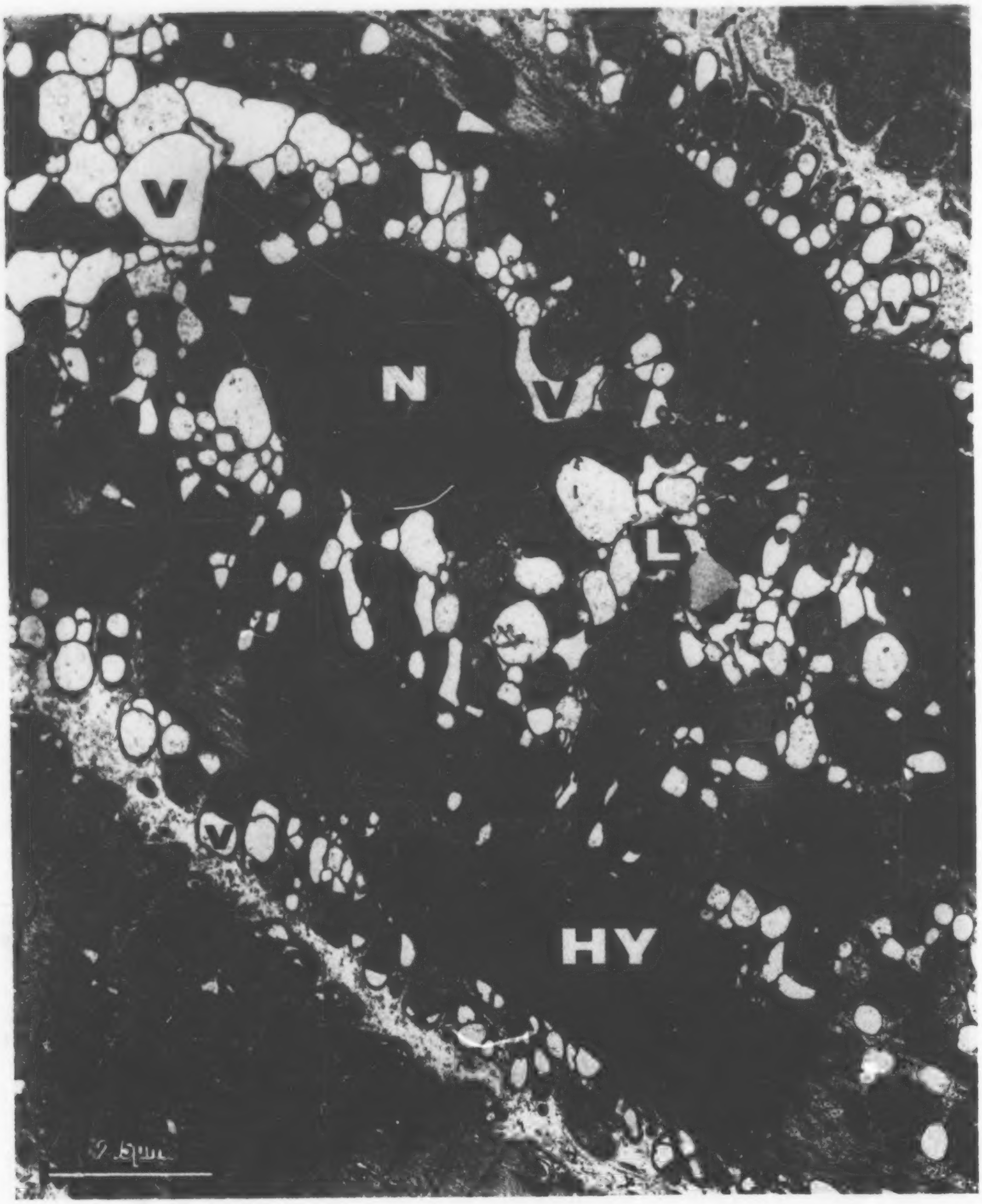

FIGURE 7.—Electron micrograph of first heart biopsy from male dog that received 0.25 mg/kg of mitoxantrone (cumulative dose of 21 mg/m^2), showing a myocyte with moderate vacuolation of the sarcoplasmic reticulum. Note that vacuolation is accentuated by contraction of muscle fiber. 11,800 ×. HY = hypercontracted myofibril; L = lipofuscin deposit; M = mitochondrion; N = nucleus of myocyte; V = vacuole derived from the sarcoplasmic reticulum; Z = Z band of myofibril.

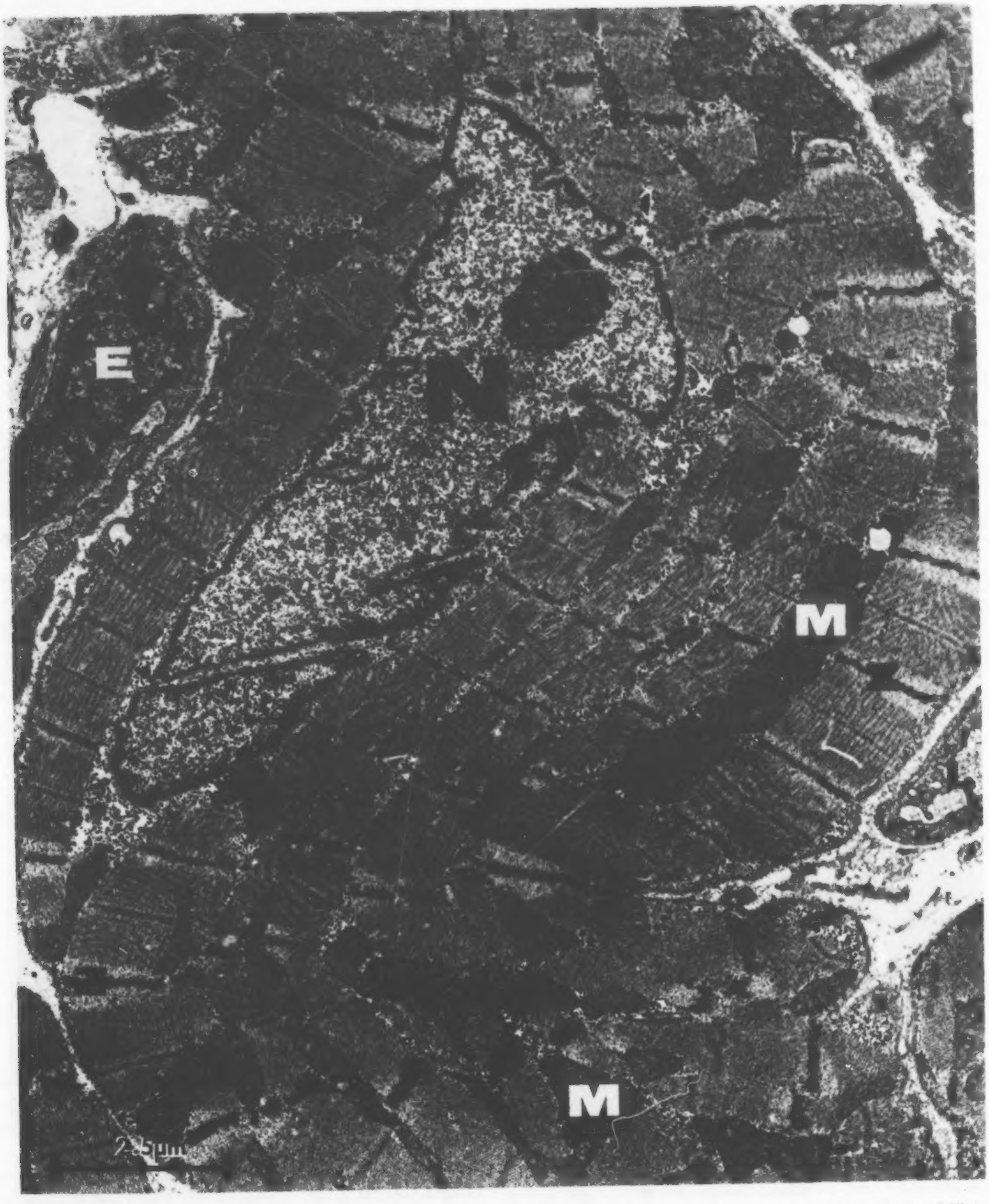

FIGURE 8.—Electron micrograph of heart sample taken postmortem from same dog as sample shown in fig 7. No evidence of dilatation of sarcoplasmic reticulum. 11,800 ×. E = endothelial cell nucleus; M = mitochondrion; N = nucleus of myocyte; Z = Z band of myofibril.

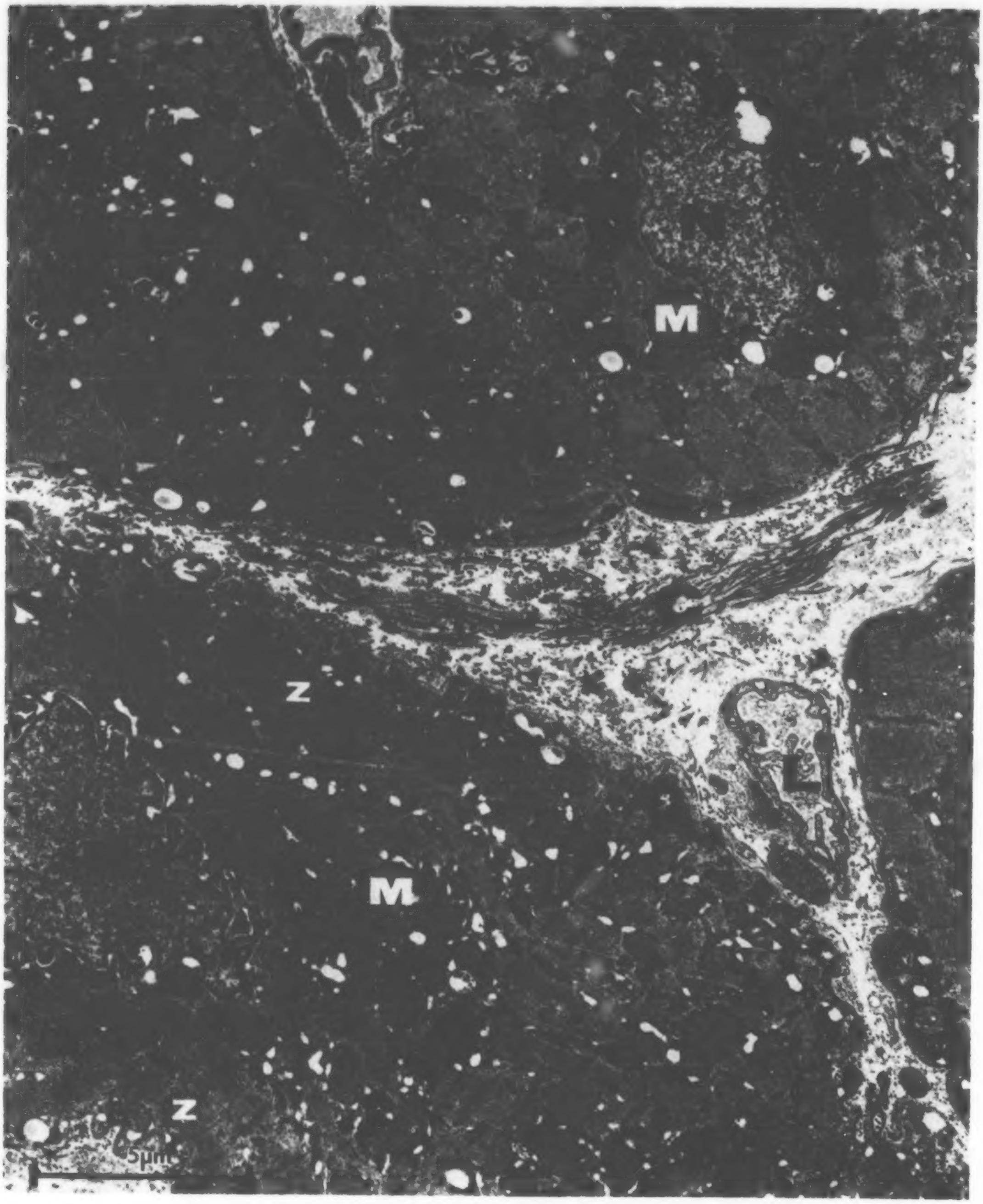

FIGURE 9.—Electron micrograph of first heart biopsy from same saline-treated dog as sample shown in fig 6. Arrows indicate dilatation of sarcoplasmic reticulum. 7200 ×. C = collagen; L = lumen of small blood vessel; M = mitochondrion; N = nucleus of myocyte; Z = Z band of myofibril.

limiting cardiomyopathy would occur with continued administration beyond 30 weeks needs to be investigated.

Degeneration of seminiferous tubules and depletion of lymphohemopoietic tissues associated with decreases in circulating leukocytes (5) are not unexpected findings with compounds known to affect rapidly dividing cells. Doxorubicin, in contrast to mitoxantrone, caused hair loss in dogs (5), which was associated with microscopically observed atrophy of hair follicles.

In a study conducted in rabbits treated with NSC 299195, the diacetate salt of mitoxantrone (13), acute cardiotoxic effects appeared to be related to severe myelosuppression and renal lesions. In their discussion, the authors did not mention that many of the rabbits, all of which died after about 6 weeks of treatment with 0.8 mg/kg of NSC 299195 once weekly, showed signs of severe septicemia, possibly associated with the immunosuppressive action of excessive doses of NSC 299195. Whether or not the acute cardiotoxicity observed in rabbits from the National Cancer Institute (NCI) study is related to the chronic cardiotoxicity observed in patients treated with anthracyclines requires further investigation. Possibly, a study in rabbits given lower doses of NSC 299195 for > 6 weeks might provide a more suitable animal model for anthracycline-like chronic cardiotoxicity. The design of such a study would allow for adequate survival of animals in the absence of severe myelosuppression, renal damage, or septicemia, all of which complicate the assessment of cardiotoxicity.

The results of the NCI-sponsored studies in rabbits treated with NSC 299195 (13) contrast with the results of the present study in which dogs were treated with the maximum tolerated dose of mitoxantrone and did not develop cardiomyopathy or clinical signs of cardiotoxicity, even in the presence of a moderate leukopenia comparable to that seen in dogs given doxorubicin (5). In addition, dogs given doxorubicin developed morphologic changes in the heart, which preceded clinical manifestations of cardiotoxicity in the absence of severe renal damage or myelosuppression.

In a companion study sponsored by NCI (14), the diacetate salt of an anthracenedione unrelated to mitoxantrone (NSC 287513) was administered to rabbits at a dose of 4 mg/kg once weekly and was compared to doxorubicin administered at a dose of 2 mg/kg weekly. Rabbits given NSC 287513 showed fewer signs of toxicity than rabbits given doxorubicin. Although there were no changes in serum parameters or ECGs in NSC 287513-treated rabbits, minimal morphologic changes observed after 12 weeks progressed to moderate or severe changes thereafter. Only rabbits treated for > 12 weeks showed signs of severe renal lesions or severe myelosuppression. In the present study, neither doxorubicin nor mitoxantrone showed evidence of severe myelosuppression or renal damage that would complicate the evaluation of cardiotoxicity seen in doxorubicin-treated dogs.

In our opinion, the chronic, progressive, and often delayed cardiotoxicity associated with anthracycline treatment must be distinguished from the acute and subacute early cardiotoxicity in patients described by Bristow et al (15) and in the animal models of Lenaz and Page (16). At this time, the relationship between acute, subacute, and chronic anthracycline-associated cardiotoxicity is unknown and deserves further study in animal models. In the present study, no signs of acute or subacute cardiotoxicity were observed in dogs given doxorubicin or mitoxantrone.

Based on doses administered, mitoxantrone appears to be about seven times more toxic than doxorubicin in the dog (the maximum tolerated dose of mitoxantrone in the dog is 0.25 mg/kg compared to 1.75 mg/kg of doxorubicin) (5) and six times more toxic than doxorubicin in man, as shown by clinical data indicating that the maximum tolerated dose of mitoxantrone in patients is 12 mg/m^2 compared to 75 mg/m^2 of doxorubicin (17). (Incidentally, the clinical doses convert to approximately 0.3 mg/kg of mitoxantrone and 1.9 mg/kg of doxorubicin, which approximate the maximum tolerated doses for several species. These data suggest that further investigation is required on the relationship of doses expressed on the basis of surface area.) Comparisons should be made on the basis of efficacy as well as toxicity. In their discussion of the activity of mitoxantrone, Wallace et al (1) stated that comparison of mitoxantrone and doxorubicin on an equimolar basis showed that mitoxantrone was approximately seven times more potent than doxorubicin in inhibiting the uptake of tritiated uridine and four times more potent in inhibiting the uptake of tritiated thymidine by mouse melanoma L5178Y cells in vitro. Since animal models have demonstrated an equivalent therapeutic efficacy highly predictive of clinical efficacy, mitoxantrone should be effective in patients at one sixth the dose of doxorubicin. The available data suggest that the relative potency of mitoxantrone is four to seven times that of doxorubicin, while its relative toxicity is seven times greater than doxorubicin.

In addition, the results of a recent study in rats (18) have shown that the parent compound of mitoxantrone has a margin of safety about twice that of doxorubicin, when the margin of safety is expressed in terms of cardiotoxic doses in rats compared to doses effective against tumors in mice. The authors did not give details of the methods used to evaluate cardiotoxicity in rats.

The results of the present study and those of Gralla et al (3), Van Vleet et al (4), and Herman and Ferrans (12) show that the dog is a useful and highly reproducible model for assessing the cardiotoxic effects of anthracyclines. Our study has added another dimension to this assessment, ie, monitoring the progression of myocardial lesions in the dog by means of sequential transvenous endomyocardial biopsies, similar to the method used in patients (2). Anthracycline cardiomyopathy occurred in the absence of severe myelosuppression, renal damage, or septicemia, which compromise the health of the animals and complicate the assessment of cardiotoxicity in other species. Mitoxantrone administered at doses equitoxic to doxorubicin did not show cardiotoxic effects in the dog.

ADDENDUM

Recently, there have been clinical reports of an obscure cardiotoxicity in patients undergoing mitoxantrone therapy (23 of approximately 1000 cases reported between April and September 21, 1981). We believe that it would be premature to discuss these cases in view of the fact that all but three of the patients had received prior anthracycline therapy. These data are, at best, equivocal, and a more definitive evaluation must await data collected under present NCI guidelines for monitoring cardiac function prior to and during mitoxantrone therapy (Department of Health and Human Services letter, July 14, 1981, to all mitoxantrone investigators).

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Increased Relative Effectiveness of Doxorubicin Against Slowly Proliferating Sarcoma 180 Cells After Prolonged Drug Exposure[1]

Paul S. Ritch,* Sandra J. Occhipinti, Karen S. Skramstad, and Stanley E. Shackney[2]

SUMMARY

Doxorubicin (ADR) lethality was studied in Sarcoma 180 cells grown in vitro during log phase and early plateau phase. For both log phase and plateau phase cells, ADR lethality increased progressively with continuous drug exposure, and was dependent on drug concentration at every point in time. For any given level of toxicity to log phase cells, greater killing of plateau phase cells was achieved with prolonged drug exposure. The inhibition of DNA synthesis by ADR was delayed in onset, and increased gradually, both at low and high drug concentrations. The data suggest that the dependence of ADR lethality on proliferation rate and the higher therapeutic ratio in plateau phase cells with prolonged drug exposure are not directly related to the ADR effects on DNA synthesis.

[Cancer Treat Rep 66:1159–1168, 1982]

Doxorubicin (ADR) is an anthracycline antibiotic that has cytotoxic activity against a broad range of experimental and human tumors (1,2). ADR is known to enter cells rapidly (3,4), localize in the nucleus (3,4), and bind to DNA (4–6) by intercalation. It has been shown to interfere with DNA polymerase (4,7–9) and DNA-dependent RNA polymerase (4,8,9), resulting in inhibition of both DNA and RNA synthesis (4,8–13). DNA strand breaks and chromosome aberrations following ADR have been observed in a variety of systems (14–16). Painter (17) showed that ADR inhibits DNA synthesis primarily by slowing the rate of chain elongation, with only a small effect on replicon initiation. Despite the many known biochemical effects of this drug, the mode of action that is directly responsible for drug lethality has not been clearly identified.

ADR lethality is dependent on drug concentration and duration of drug exposure (10,18–22), and ADR has also been shown to be more effective against log phase cells than against plateau phase cells in a variety of cell systems (18,19,22). However, the relationship between this phenomenon and known effects of ADR on DNA synthesis is not clear. Published reports on ADR cycle phase specificity have been conflicting. It has been reported that cells are maximally sensitive to ADR during S phase (10,23), during mitosis and early S phase (24), and during late S phase and G_2 (20) and that ADR induces cell cycle traverse delay with progression blockade in G_1 (25–27), in S phase (14,25–27), and in G_2 (14,22,23,25–27).

These studies have provided no clear recommendations regarding optimal drug scheduling. Pharmacokinetic studies have demonstrated a prolonged plasma half-life for ADR following pulse doses (28,29), and intermittent single high-dose schedules have been recommended on this basis. A plateau in the pulse dose-response curve has been reported in some experimental cell systems, prompting the suggestion of multiple intermittent low-dose schedules as optimal (30). With these considerations in mind, we undertook to study the lethal effects of ADR in Sarcoma 180 cells grown in vitro, in relation to drug concentration and drug exposure duration, and to examine the role of inhibition of DNA synthesis by ADR in producing its lethal effects.

The proliferation kinetics of this cell system have been described in detail in previous reports (31,32).

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[1] Received Dec 29, 1980; revised Nov 3, 1981; accepted Dec 16, 1981.

[2] Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to*: Paul S. Ritch, MD, Hematology-Oncology Section, Department of Medicine, The Medical College of Wisconsin, 8700 W Wisconsin Ave, Milwaukee, WI 53226.

To summarize briefly, percent labeled mitosis curve studies have shown that the majority of 2-day-old log phase cells have cell cycle times in the range of 10–16 hours. The majority of 4-day-old plateau phase cells have a cycle time in the range of 20–26 hours. The labeling index of tritiated thymidine ([^{3}H]TdR) is 0.72 in 2-day log phase cells (4 days of emulsion exposure) and decreases to 0.48 in 4-day plateau phase cells. The effective duration of S phase increases with cell cycle prolongation, and there is a twofold reduction in the maximum rate of DNA synthesis between Days 2 and 4.

MATERIALS AND METHODS

All studies were carried out in Sarcoma 180 (Foley strain CCRF11, supplied by American Tissue Type Culture, Rockville, MD) grown in vitro in Earle's Medium 199 (Flow Laboratories, Rockville, MD), which was supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. Cultures were grown in monolayer in 250-ml plastic tissue culture flasks (growth surface area, 75 cm^2) (Costar, Cambridge, MA) containing 10 ml of medium. Cells were plated at an initial concentration of 1 × 10^5 cells/ml. Medium was changed on Days 2 and 4, and cells were subcultured on Day 5.

Cloning Studies

Two-day-old log phase cell cultures and 4-day-old early plateau cultures were incubated (37°C) with ADR at final concentrations ranging from 0.1 to 4.0 μg/ml for 1 hour and at final concentrations ranging from 0.05 to 0.5 μg/ml for 24 hours. At the end of the drug exposure period, the medium containing drug was removed and the cells were rinsed four times with 5 ml of Hanks' balanced salt solution (HBSS). Cells were harvested by incubation (37°C) with 0.25% trypsin for 8 minutes. All drug exposures were carried out in duplicate, and duplicate controls were obtained at each time point. Total cell counts were determined using a Coulter counter. Cell suspensions were diluted with Medium 199, and known numbers of cells were cloned in soft agar. Nine-day-old colonies were fixed, stained with Giemsa stain, and counted with the aid of a magnifying lens. Control colonies ranged from 1 to 5 mm in diameter; drug-treated colonies ranged from 0.5 to 2 mm in diameter. Small colonies were examined microscopically, and only those containing $\geq$ 20 cells were counted. Clonogenic cell yield—defined as the number of colonies enumerated divided by the number of cells plated—was averaged for five replicate flasks at each concentration. Clonogenic cell yields for controls were approximately 60% for log phase cells and 40% for plateau phase cells. The viable cell count per flask was calculated as the total cell number per flask times the clonogenic cell yield at a given time point. The surviving viable cell fraction following drug exposure was calculated as the number of viable drug-treated cells per flask divided by the number of viable untreated control cells per flask. Each experiment was performed at least twice, and the reported results represented the log means of replicate studies.

[^{3}H]TdR Incorporation

Cells were harvested at intervals following drug exposure. Cultures were exposed to [^{3}H]TdR (specific activity, 6.7 curies/mmol; New England Nuclear, Boston, MA) at a final concentration of 2.0 microcuries/ml for 30 minutes prior to sample collection. Cells were washed three times with HBSS containing 10^{-5} M cold TdR, harvested by trypsinization, and counted. A 1-ml aliquot was washed once with 0.85% NaCl, centrifuged at 400 g for 15 minutes, and resuspended in 10 ml of 10% trichloroacetic acid. Cell precipitates were collected on a 0.45-μ Millipore filter with extensive trichloroacetic acid washing and placed in counting vials to which 17 ml of Aquasol (New England Nuclear) was added for scintillation counting. [^{3}H]TdR incorporation (disintegration per minute/10^6 cells) in all drug-treated flasks was expressed as the fraction of untreated control. Each study was performed at least twice, and the results reported represent the log means of the replicate data at each time point.

DNA Content Distribution Studies

DNA content distributions were measured serially following 1-hour exposure and during continuous 24-hour exposure of ADR at high and low drug concentrations. Cells were washed with HBSS, fixed in cold 70% ethanol, and stained with 100 μg/ml of mithramycin in 15 mM $MgCl_2$ and 0.15 M NaCl at a final cell concentration of 1 × 10^6 cells/ml. Nuclear fluorescence was measured with a Los Alamos Cell Sorter. Data were recorded, stored, and analyzed on a DEC 11/40 computer system, using software developed at the Los Alamos Scientific Laboratory, NM. All DNA content distributions were obtained at constant photomultiplier current and amplifier gain settings. At least 50,000 cells were measured in each sample. All DNA content distributions were normalized with respect to total cells analyzed per sample for display purposes.

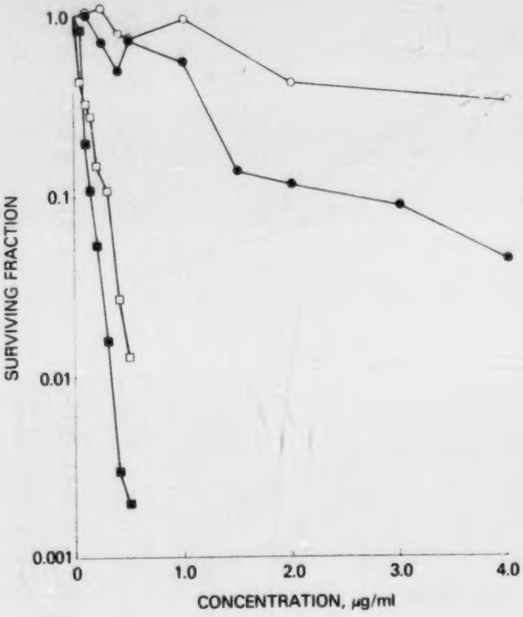

FIGURE 1.—Effects of ADR on cell viability in relation to drug concentration and drug exposure duration. 1-hr exposure: log phase cells, ●; and early plateau phase cells, ○. 24-hr exposure: log phase cells, ■; and early plateau phase cells, □. Drug exposure times are from start of drug exposure. For discussion, *see* text.

RESULTS

Cell Cloning Studies

The effects of drug concentration and duration of drug exposure on cell survival are shown in figure 1. As ADR concentration is increased, fewer clonogenic cells survive. Following brief exposure, fractional cell kills approaching 0.9 are achieved only at concentrations > 1 μg/ml and only in log phase cells. Following prolonged drug exposure, fractional cell kills approaching or > 0.99 are achieved at drug concentrations of 0.4 μg/ml in both log phase and early plateau phase cells.

The time course of ADR lethality during prolonged drug exposure at drug concentrations of 0.1 and 0.4 μg/ml is shown in figure 2. There is a progressive decrease in the surviving cell fraction in both log phase (fig 2A) and early plateau phase (fig 2B) cells with increasing duration of drug exposure. ADR lethality was dependent on drug concentration at every point in time.

Figure 3 compares the relative effectiveness of high-dose, brief exposure and low-dose, prolonged exposure in killing plateau phase cells. To construct the curves, the log kill obtained in early plateau phase cell cultures following a 1-hour drug exposure was compared to that following a 24-hour exposure under conditions of drug exposure that produced the same log kill in log phase cells (fig 1). For example, following a 1-hour drug exposure, the concentration of ADR that produced a log kill of 1 in log phase cells produced approximately a 0.3 log kill in early plateau cells. Following a 24-hour drug exposure, the concentration of ADR that produced a 1 log kill in log phase cells produced approximately a 0.6 log kill in early plateau phase cells. These comparisons were carried out at various levels of effectiveness against log phase cells (fig 3A). The fact that all points on the curve for the 24-hour schedule lie above those of the 1-hour schedule indicates that, *for any given level of effectiveness against log phase cells*, ADR was more effective against early plateau phase cells when given by low-dose, prolonged exposure than by high-dose, brief exposure.

To determine whether the *degree* of relative effectiveness against early plateau phase cells obtained by prolonged drug exposure changed with treatment intensity, the therapeutic index (defined as fractional cell kill obtained in early plateau phase cells/fractional cell kill in log phase cells) was plotted as a function of log kill in log phase cells. For drug schedules that produce log kills ranging from 0.5 to 1.5 in log phase cells, the relative advantage of prolonged exposure over brief exposure in plateau phase cells was unchanged (fig 3B).

[^{3}H]TdR Incorporation Studies

Log phase cells were incubated with ADR at final concentrations of 0.5 and 2.0 μg/ml for 1 hour and at 0.1 and 0.4 μg/ml for 24 hours. The suppression of [^{3}H]TdR incorporation following brief exposure to ADR was delayed and occurred gradually, both at low and high drug concentrations (fig 4A). At the higher drug concentration, there was progressive suppression of [^{3}H]TdR incorporation over the entire period of observation. At the lower drug concentration, recovery to pretreatment levels of [^{3}H]TdR incorporation was observed at 24 hours.

The effects of continuous ADR exposure on [^{3}H]TdR incorporation are shown in figure 4B. In the continued presence of ADR, suppression of [^{3}H]TdR incorporation was also delayed and occurred gradually, both at low and high drug concentrations.

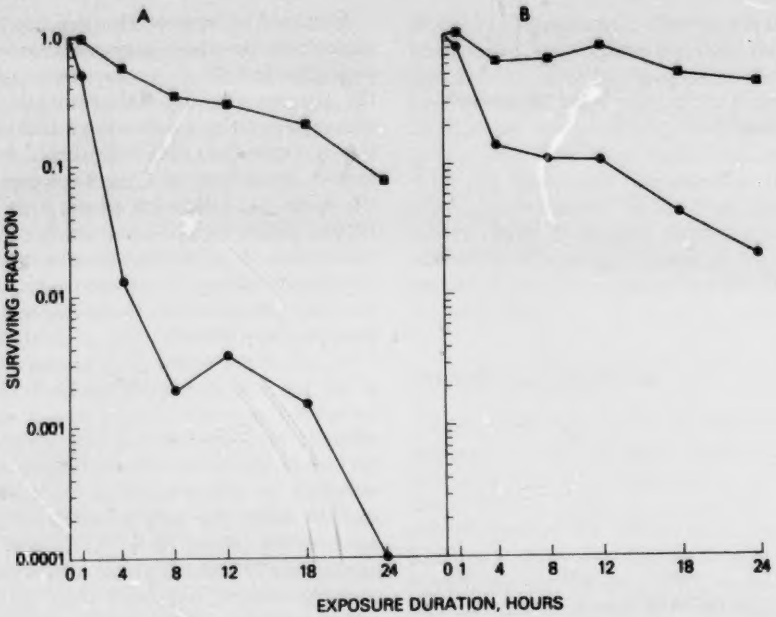

FIGURE 2.—A: time course of ADR lethality in log phase culture. Surviving cell fractions at ADR concentrations of 0.1 μg/ml, ■; and 0.4 μg/ml, ●. B: time course of ADR lethality in early plateau phase culture. Surviving cell fractions at ADR concentrations of 0.1 μg/ml, ■; and 0.4 μg/ml, ●. All drug exposure times are from start of drug exposure. For discussion, *see* text.

Flow Cytometry Studies

The effects of ADR on DNA synthesis were also studied by flow cytometry. The changes in the DNA histogram of log phase cells following exposure to 0.5 μg/ml or 2.0 μg/ml of ADR for 1 hour are shown in figure 5. There was a decrease in the fraction of cells in the G_1 and early S regions and an increase in the fraction of cells in the late S and G_2M regions at 8 hours (fig 5, compare B3 and C3 with A3). The timing and magnitude of these changes were independent of drug concentration for the first 8 hours. A further decrease in the fraction of cells in the G_1 and early S regions was observed at 16 hours at both drug concentrations (fig 5, B4 and C4). However, following exposure to the lower drug concentration, there was an increase in the height of the G_1 peak at 16 hours, indicating progression through mitosis. This was followed by a continued increase in the fraction of cells in the early S region of the DNA histogram at 24 hours (fig 5, B5), indicating the resumption of active DNA synthesis. This paralleled the recovery that was observed in [^{3}H]TdR incorporation (fig 4A). At the higher drug concentration, there was no appreciable change in the DNA content distribution between 16 and 24 hours (fig 5, compare C5 with C4), in agreement with the decrease in [^{3}H]TdR incorporation to 1% of the control value at 24 hours (fig 4A).

The changes in the DNA histogram during prolonged exposure of log phase cells to 0.1 or 0.4 μg/ml of ADR are shown in figure 6. The changes observed during the first 8 hours were similar to those observed following exposure to higher pulse doses and were also independent of drug concentration. There was a decrease in the fraction of cells in the G_1 and early S regions and an increase in the fraction of cells in the late S and G_2M regions of the DNA histogram (fig 6, B3 and C3). There was continued clearing of cells from the G_1 and early S regions with time, and most cells were found in the late S and G_2M regions of the DNA histogram at 24 hours (fig 6, B3–6 and C3–6). There was no appreciable change in the DNA histogram between 18 and 24 hours. [^{3}H]TdR incorporation at this time was approximately 1% of control (fig 4B).

The changes in the DNA histogram during prolonged exposure of plateau phase cells to ADR are

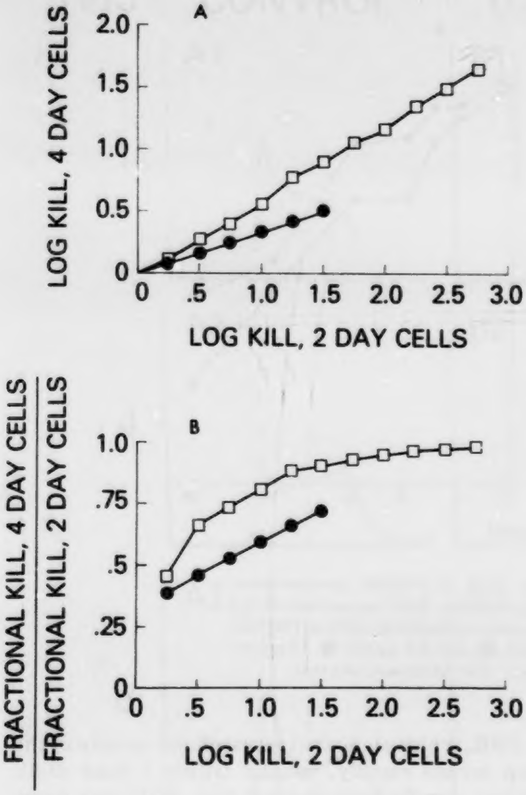

FIGURE 3.—A: relative toxicity of ADR to log phase cells and early plateau phase cells: high-dose, 1-hr exposure, ●; and low-dose, 24-hr exposure, □. B: therapeutic ratio (defined as fractional cell kill obtained in early plateau phase cells/fractional cell kill in log phase cells) at different levels of treatment intensity: high-dose, 1-hr exposure, ●; and low-dose, 24-hr exposure, □. Log kill = −log (surviving cell fraction). For discussion, *see* text.

shown in figure 7. Differences between ADR-treated cells and controls were apparent at 18 hours and were qualitatively similar to those seen at 8 hours in log phase cells (fig 6). The magnitude and subsequent time course of these changes were similar at both the 0.1- and 0.4-μg/ml concentrations.

DISCUSSION

Most treatment-limiting toxic effects experienced by patients receiving chemotherapy with ADR result from damage to rapidly proliferating tissues, such as bone marrow and the mucosal cells of the alimentary tract. One might expect that these tissues would be severely affected by ADR, regardless of how the drug is administered. Under these circumstances, the objective of drug schedule optimization should be to maximize the lethal effects of ADR in slowly proliferating tumors for any given level of toxicity to rapidly proliferating host tissues. Barranco (30) showed that a fractionated low-dose technique is more effective at reducing cell survival than larger single doses and suggested that toxic effects might be reduced by this schedule. Our studies suggest that prolonged exposures to ADR at low drug concentrations achieve this objective better than brief exposures at high drug concentrations. Although rapidly proliferating cells are more sensitive to ADR than slowly proliferating cells under both drug exposure conditions, prolonged drug exposure produces *greater cell killing of slowly proliferating cells* than a 1-hour exposure under conditions that are equitoxic to rapidly proliferating cells.

The clinical usefulness of these findings remains to be determined. There are many factors that affect the clinical therapeutic index; these include differences in cell kill between tumor and normal host cells, in the rates of regrowth of tumor and normal host cells following exposure to cytotoxic agents, in drug pharmacokinetics, and in drug metabolism in different tissues. In our experimental cell system we have examined only one of these factors, namely, cell kill that is dependent on proliferation rate. Since we did not examine population regrowth or pharmacologic and pharmacokinetic factors in our studies, we cannot predict with certainty whether prolonged infusions of ADR will be of clinical benefit on the basis of our experimental data alone. However, given that the hair matrix-forming cells, the mucosal cells of the alimentary canal, and the hematopoietic bone marrow cells are among the most rapidly proliferating cells in the body and given that the last two are responsible for treatment-limiting, short-term toxic effects in the host, it would seem reasonable to expect that prolonged ADR infusion may result in a greater effect against slowly proliferating tumors than pulse exposure, for a given level of host toxicity.

Preliminary studies in man have shown prolonged ADR infusions to have antitumor activity (33,34). The studies of Legha et al (33) suggest that ADR given by prolonged infusion is at least as effective in the treatment of advanced breast cancer as when given by single iv injection. In view of our findings, additional studies in this area are warranted.

If prolonged infusions prove to be relatively more effective clinically against slowly proliferating tumors, it is conceivable that slowly proliferating normal host tissues might also be damaged to a greater degree. However, this possibility has not been borne

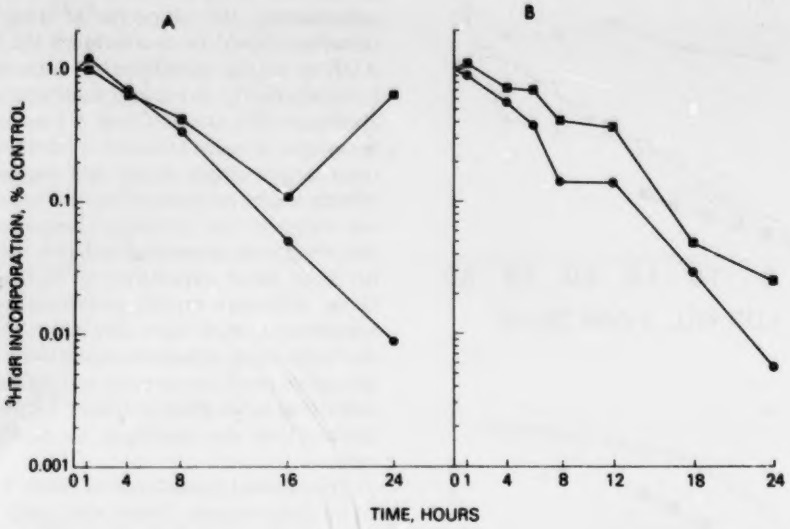

FIGURE 4.—A: effects of a 1-hr exposure to ADR on [^{3}H]TdR incorporation. Abscissa shows time from initiation of drug exposure. ADR concentration: 0.5 μg/ml, ■; and 2.0 μg/ml, ●. B: effects of continuous exposure to ADR on [^{3}H]TdR incorporation. ADR concentration: 0.1 μg/ml, ■; and 0.4 μg/ml, ●. Drug exposure times are from start of drug exposure. For discussion, *see* text.

out in the clinical data that are available to date; if anything, cardiac toxicity may be less with prolonged, low-dose ADR administration (33).

Our studies showed that the inhibition of [^{3}H]TdR incorporation following pulse ADR exposure was delayed and occurred gradually. Gradual suppression of [^{3}H]TdR incorporation following exposure to ADR has been observed previously in some experimental systems (7,12) but not in others (4,9–11). However, in flow cytometry studies, the ability of cells to traverse the S region and accumulate in the late S–G_2M region of the DNA histogram has been a universal finding (22,23,25–27). In our studies, cells exposed to ADR were able to synthesize a full or nearly full complement of DNA and accumulated in the late S–G_2M region of the DNA histogram regardless of drug concentration or schedule of administration, both in log phase and plateau phase cultures (figs 6 and 7). In contrast, the lethal effects of ADR were highly dependent on drug concentration and schedule of administration, both in log phase and plateau phase cells (figs 3A and B). Thus, it is unlikely that inhibition of DNA synthesis is directly responsible for the lethal effects of ADR.

It is conceivable that the apparent delay in inhibition of DNA synthesis could be due to delayed intracellular uptake of ADR. However, this is not consistent with published pharmacokinetic studies of ADR, which show that intracellular uptake of the drug occurs rapidly, usually within 1 hour (3,4). Rather, our findings suggest that ADR may exert its lethal effects on some metabolic process other than DNA synthesis and that the inhibition of DNA synthesis may be a secondary effect. Dethlefsen and Riley (35) demonstrated that ADR administered in vivo maximally exhibited RNA synthesis in murine duodenal mucosa between 2 and 6 hours, whereas DNA synthesis was not maximally suppressed until 16 hours after injection. It has been shown in Sarcoma 180 ascites tumor (13) and HeLa S-3 cells (11) that ADR inhibits RNA synthesis to a greater extent than DNA synthesis; in other experimental systems, both processes are inhibited to a similar degree (8,9,12). Rapidly proliferating cells have been shown to have higher RNA contents than slowly proliferating cells (36). It is possible, then, that higher rates of RNA synthesis in rapidly proliferating cells could render them more vulnerable to the lethal effects of ADR than slowly proliferating cells. Clearly, additional studies are required to explore this possibility.

In summary, our studies suggest that the relative therapeutic effectiveness of ADR against slowly proliferating cells may be improved by administering the drug as a prolonged infusion and that the lethal effects of ADR are probably not directly re-

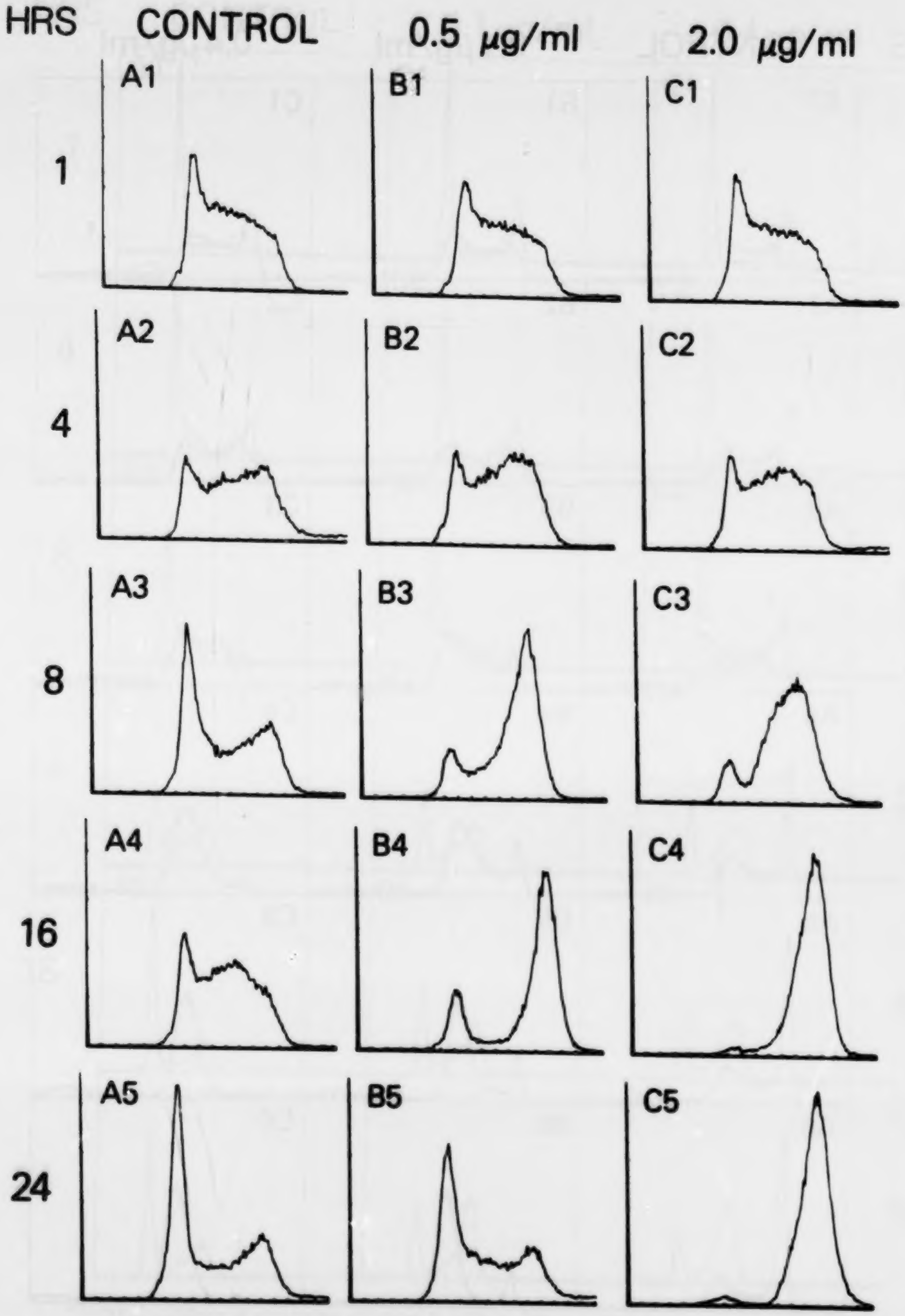

FIGURE 5.—Effects of a 1-hr exposure to ADR on DNA content distribution of log phase cells. A1–A5, control cultures; B1–B5, 0.5 μg/ml of ADR; and C1–C5, 2.0 μg/ml of ADR. All DNA histograms are normalized with respect to total cells measured per sample, and ordinate scale is identical for all histograms. DNA/cell is shown on abscissa, and frequency is displayed on ordinate.

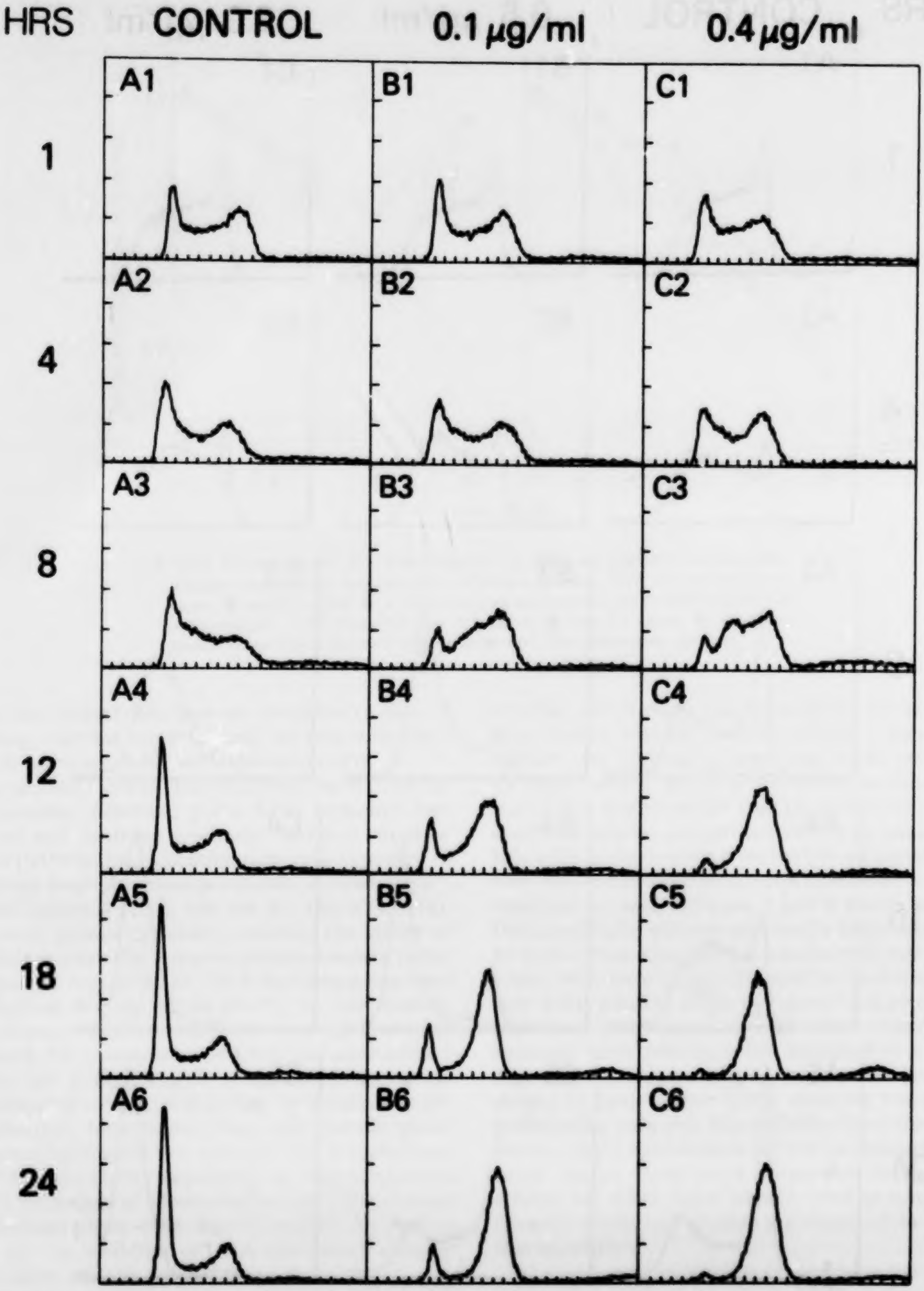

FIGURE 6.—Effects of continuous exposure to ADR on DNA content distribution of log phase cells. A1–A6, control cultures; B1–B6, 0.1 μg/ml of ADR; and C1–C6, 0.4 μg/ml of ADR. All DNA histograms are normalized with respect to total cells measured per sample, and ordinate scale is identical for all histograms. DNA/cell is shown on abscissa, and frequency is displayed on ordinate.

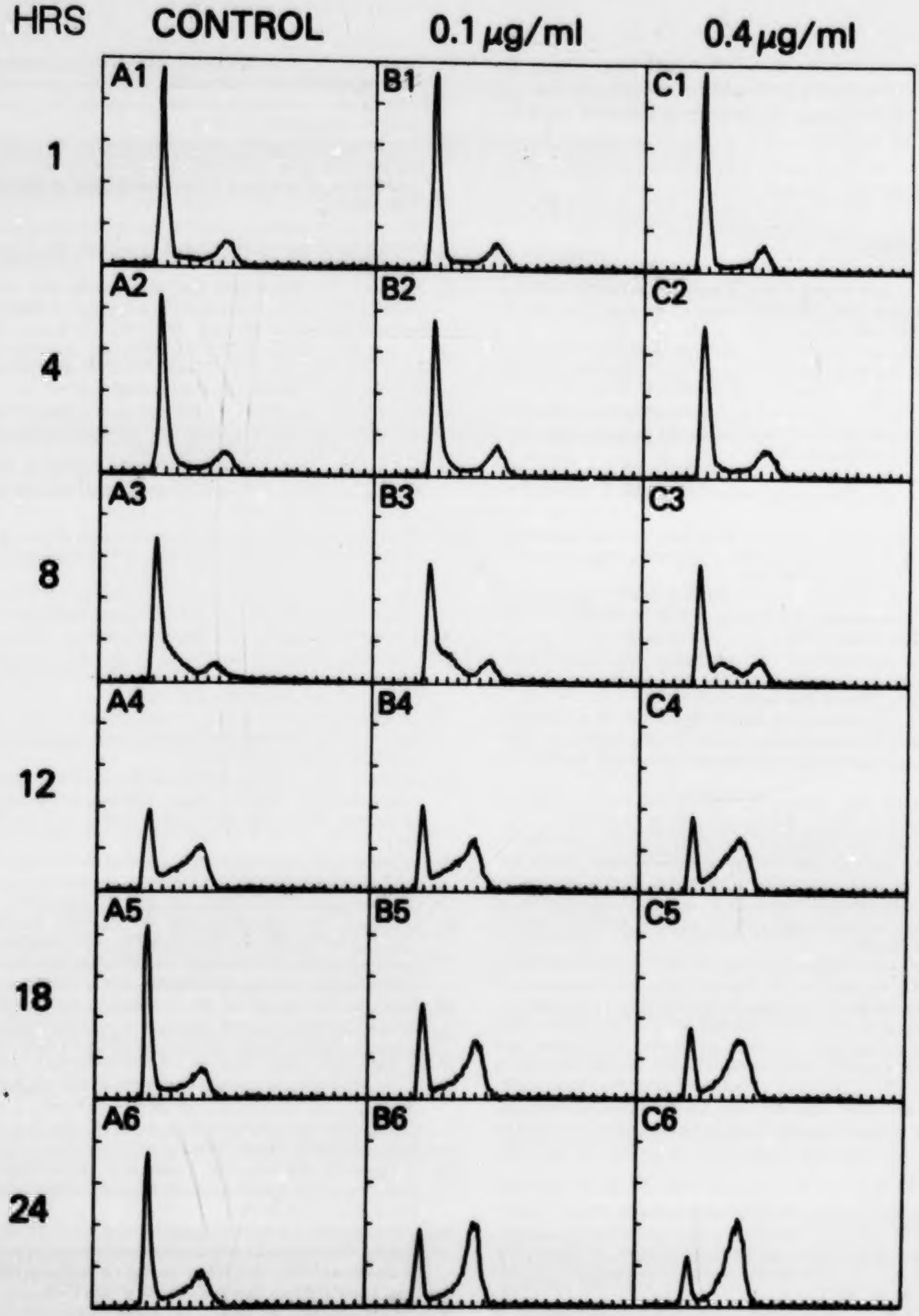

Figure 7.—Effects of continuous exposure to ADR on DNA content distribution of early plateau phase cells. A1–A6, control cultures; B1–B6, 0.1 μg/ml of ADR; and C1–C6, 0.4 μg/ml of ADR. All DNA histograms are normalized with respect to total cells measured per sample, and ordinate scale is identical for all histograms. DNA/cell is shown on abscissa, and frequency is displayed on ordinate. For discussion, *see* text.

lated to its inhibition of DNA synthesis. Thus, an alternative mechanism should be sought for the increased effectiveness of the drug against rapidly proliferating cells, as well as for its greater therapeutic ratio against slowly proliferating cells, with prolonged drug exposure.

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Effect of Activated Charcoal on the Pharmacokinetics of High-Dose Methotrexate[1,2]

S. D. Gadgil,[3,*] S. R. Damle,[4] S. H. Advani,[4] and A. B. Vaidya[3,5]

SUMMARY

The serum methotrexate (MTX) concentrations were estimated in two groups of patients after 6-hour iv infusion of 1 g/m^2 of MTX and leucovorin rescue. One group (seven patients) received 25 g of activated charcoal orally at 12, 18, 24, 36, and 48 hours, while the other group served as a control (eight patients). Pharmacokinetic analysis revealed a significant reduction in the area under concentration curve from 18 hours until the serum MTX levels reached 10^{-8} M. The MTX concentrations were significantly lower in the group that received charcoal than in the control group, at 18, 24, 36, 48, 60, and 72 hours from the end of infusion. First and second half-life values for the control and the charcoal-treated groups did not differ significantly. The data suggest that charcoal aids in the elimination of MTX from the body by interruption of the enterohepatic circulation of the drug. Activated charcoal did not cause any undesirable effects.

[Cancer Treat Rep 66:1169–1171, 1982]

High-dose methotrexate (MTX) regimens with leucovorin rescue have become a part of many chemotherapy protocols (1). Administration of leucovorin is scheduled during the last phase of MTX decay (the "tail" part of the biexponential curve), which is responsible for the toxicity to the bone marrow and the mucosa of the gastrointestinal tract (2,3). This slow second phase of MTX elimination appears to be due to nonrenal mechanisms (ie, biliary and gut clearance mechanisms) (1). Recently, Neuvonen and Elonen (4) have demonstrated that orally administered activated charcoal is efficacious in accelerating intestinal elimination of several drugs (eg, phenobarbital and carbamazepine). The present communication reports observations on the pharmacokinetics of iv high-dose MTX with oral doses of activated charcoal.

MATERIALS AND METHODS

A total of 15 patients received high-dose MTX for adjuvant treatment of osteogenic sarcoma (12 patients); carcinoma of the breast (one) or head and neck (one); and soft tissue sarcoma (one). Their ages ranged from 14 to 60 years. All patients received high-dose MTX after excision of the primary tumor. Eight control patients (group 1) and seven charcoal-treated patients (group 2) were matched for tumor type and age; renal and hepatic functions were within normal limits.

MTX (1 g/m^2) was infused iv over a period of 6 hours, followed by leucovorin rescue (9–15 mg/m^2 im) given every 6 hours between 6 and 60 hours after the end of MTX infusion. Alkalization of urine was not done. A single dose of activated charcoal powder (25 g) was given orally as a suspension in

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[1] Received Apr 10, 1981; revised Oct 29, 1981; accepted Nov 17, 1981.

[2] CIBA-GEIGY Research Centre (CGRC) Contribution No. 621.

[3] Department of Clinical Research, CGRC, Bombay, India.

[4] Departments of Medicine and Chemotherapy, Tata Memorial Hospital, Bombay, India.

[5] We thank Dr. Praful B. Desai, Director, Tata Memorial Hospital, Bombay, for permission to conduct the study and Prof. D. Subrahmanyam, Director, CGRC, for guidance and encouragement, and we acknowledge the help of Dr. S. C. Bhatia, CGRC, in pharmacokinetic analysis.

[*] *Reprint requests to:* S. D. Gadgil, MD, Medical Research Division, Pfizer Ltd, Express Towers, Nariman Point, Bombay 400 021, India.

TABLE 1.—MTX serum concentrations (mols/liter)*

Hrs	Concentration		*P* value
	Group 1†	Group 2‡	
0	$7.60 \times 10^{-5} \pm 0.45$	$7.3 \times 10^{-5} \pm 0.54$	NS
3	$1.56 \times 10^{-5} \pm 0.20$	$2.00 \times 10^{-5} \pm 0.15$	NS
12	$1.18 \times 10^{-6} \pm 0.23$	$1.48 \times 10^{-6} \pm 0.11$	NS
18	$5.8 \times 10^{-7} \pm 0.74$	$3.18 \times 10^{-7} \pm 0.58$	< 0.02
48	$4.01 \times 10^{-8} \pm 0.46$	$1.67 \times 10^{-8} \pm 0.16$	< 0.001
72	$1.35 \times 10^{-8} \pm 0.73$	$0.38 \times 10^{-8} \pm 0.09$	< 0.001

*Values = mean ± SE. NS = not significant.
†Control group (N = 8).
‡Charcoal-treated group (N = 7).

300 ml of water at 12 or 18 hours and was repeated at 24, 36, and 48 hours after the end of MTX infusion. The pharmacokinetics of MTX was studied in one patient with and without charcoal.

Blood samples were drawn at 0, 1, 3, 6, 12, 18, 24, 36, 48, 60, and 72 hours after the end of MTX infusion. Serum was separated and stored frozen. Serum MTX was estimated by the modified enzyme kinetic method of Bertino and Fisher (5), as reported earlier (6). Pharmacokinetic analysis was done by biexponential fit using least-squares analysis on a programmable calculator, with the following equation:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

Half-life values were calculated by fitting individual patient's data and the mean was computed for the group. Area under the curve (AUC) was calculated from 18 hours until the concentrations reached 10^{-8} M. The area of this triangular segment of the curve was calculated using β slope and the ordinate at 18 hours. Unpaired Student's t test was employed for the statistical analysis.

RESULTS

Serum MTX concentrations for the two groups are shown in table 1. First- and second-phase half-life values for the control group were 1.95 ± 0.08 hours and 8.46 ± 0.47 hours, respectively. The corresponding values for the charcoal-treated group were 2.1 ± 0.07 hours and 7.6 ± 0.44 hours. The serum half-life values did not differ significantly between the two groups. But the serum concentrations were significantly lower in the charcoal-treated group from 18 to 72 hours. Table 2 shows the integrated AUC for individual patients from the concentration at 18 hours to 10^{-8} M. Figure 1 shows the mean serum MTX concentration against time. Concentrations up to 12 hours are superimposed for the two groups. After 18 hours, the concentrations are lower in the charcoal-treated group, with an increasing difference toward 60 hours; after 60 hours the levels are $< 10^{-8}$ M.

DISCUSSION

The observations indicate that activated charcoal given orally can lower serum MTX concentrations effectively. Though the slow-phase half-life is not reduced significantly in this study, the product of time × concentration exposure was reduced significantly by the present dose schedule of activated charcoal.

These findings support the earlier contention that enterohepatic circulation contributes to the slow phase of MTX elimination (1).

TABLE 2.—AUC for MTX from concentration at 18 hrs to 10^{-8} mols/liter

Patient No.	AUC (concentration × hr)
Group 1 (control)	
1	68.66
2	87.40
3	90.10
4	103.4
5	112.11
6	93.90
7	105.00
8*	108.62
Mean ± SE	96.15 ± 5.0†
Group 2 (charcoal-treated)	
8a*	69.06
9	67.62
10	65.36
11	69.49
12	62.14
13	50.50
14	42.70
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Mean ± SE	60.9 ± 4.0†

*Same patient, with and without charcoal.
†$P < 0.01$.

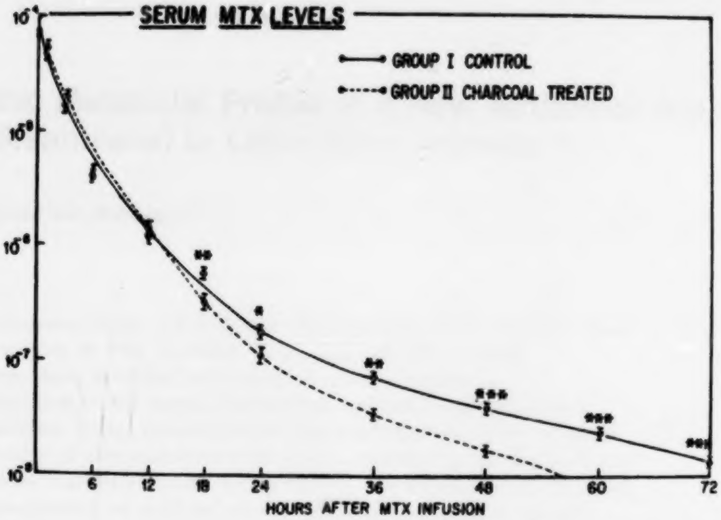

FIGURE 1.—Mean serum MTX levels (mols/liter). Vertical bars denote SE; * $P < 0.05$, ** $P < 0.02$, and *** $P < 0.001$.

Patients in both groups were taking oral fluids ad libitum. The effect of an additional 300 ml of water given two times a day with activated charcoal would be negligible if any, especially on the second phase of MTX elimination. Findings of this preliminary study indicate that enteral charcoal may help to reduce MTX toxicity by interrupting the enterohepatic circulation. This would benefit those patients who are in renal failure or have received high doses of MTX, because they enter the terminal phase of MTX disappearance with higher serum concentrations. The oral charcoal method is simple, safe, practical, noninvasive, and inexpensive compared to hemoperfusion with activated charcoal (7). Hemoperfusion is required for bulk removal of MTX from the blood, and therefore it is useful during the first part of the MTX elimination, which is a function of renal clearance. But the oral charcoal treatment may play an important role during the second part of the decay, which is the enterohepatic phase.

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Disposition and Metabolic Profile of a New Antitumor Agent: CL 216,942 (Bisantrene) in Laboratory Animals[1,2]

Wen Hui Wu and Gabriela Nicolau[3,4,*]

SUMMARY

A ^{14}C-labeled antitumor drug, CL 216,942 (bisantrene), was administered iv at doses of 1–20 mg/kg to the monkey, dog, and rat. The serum concentration versus time profiles indicated at least a biphasic disappearance of the drug with long elimination half-lives of about 2–8 days in all three species. Total recoveries in the excreta collected through Day 7 were low (< 50% of the administered dose), especially in the monkey and dog, with most of the drug found in the feces. Volumes of distribution in the central compartment as well as tissue distribution studies in all three species indicated a rapid uptake and significant concentration of the drug with long elimination half-lives in a deep compartment. The same organs and tissues (heart, liver, kidney, pancreas, spleen, lymph nodes, bone marrow, and most of the highly perfused glands) contained the highest drug concentrations in all three species. Metabolism studies indicated no evidence of significant biologic transformation of the drug in these species. In vitro studies by equilibrium dialysis indicated significant binding of the drug to DNA by intercalation.

[Cancer Treat Rep 66:1173–1185, 1982]

A new antitumor agent, CL 216,942 (bisantrene), is one of a series of anthracene-dicarboxaldehyde bis hydrazones which have been synthesized at the Medical Research Division of the American Cyanamid Company (1). The compound has demonstrated significant antitumor activity in experimental mouse tumor systems (1) and in several types of tumors investigated by an in vitro stem cell assay. It is presently undergoing clinical studies in patients with treatment-resistant malignancies, and the preliminary results are very encouraging.[5] This study was designed to determine pharmacokinetic data, tissue distribution, and metabolic fate of CL 216,942 following its administration to laboratory animals.

MATERIALS AND METHODS

Animals

The rat, the dog, and the monkey were selected for study. Two strains of male rats, Charles River (Charles River Breeding Laboratories, Wilmington, MA) and Wistar (Royal Hart Laboratories, Newhampton, NY), were used; their weight range was 190–268 g. The dogs were purebred beagles: six females (Laboratory Research Enterprises, Kalamazoo, MI) and five males (Marshal Research, North Rose, NY) with a weight range of 9–14 kg. Four

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[1] Received May 4, 1981; revised Nov 17, 1981; accepted Dec 2, 1981.

[2] Presented in part at the 20th Interscience Conference on Antimicrobial Agents and Chemotherapy in New Orleans, LA, Sept 1980.

[3] Department of Pharmacodynamics, Medical Research Division, American Cyanamid Co, Pearl River, NY.

[4] We thank D. Cosulich for mass spectral analysis, S. Gordon for tissue deposition analysis by whole-body autoradiography, D. Watts for work on high-pressure liquid chromatography assay development, and W. McWilliams for technical assistance.

* *Reprint requests to:* Gabriela Nicolau, PhD, Department of Pharmacodynamics, Medical Research Division, American Cyanamid Co, Pearl River, NY 10965.

[5] Von Hoff DD, Myers GW, Kuhn G, et al. Phase I clinical investigation of 9,10-anthracene-dicarboxaldehyde, bis(2-imidazolin-2-ylhydrazone) (CL 216,942). Submitted for publication.

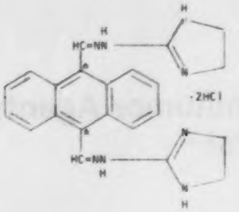

FIGURE 1.—Chemical structure of CL 216,942.

cynomolgus monkeys (Primate Import Corp, Port Washington, NY), two males and two females weighing 3–5 kg, were used. The animals were housed in stainless steel metabolism cages in air-conditioned quarters. Two of the rats were housed in special metabolism cages equipped to trap expired CO_2. All animals had free access to water, but received no food for 16 hours before and about 4 hours after dosing.

Compounds

Unlabeled and ^{14}C-radiolabeled CL 216,942 (9,10-anthracenedicarboxaldehyde bis[(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride) were synthesized at Lederle Laboratories (1). The radiolabeled compound contained two ^{14}C atoms in the 9,10 positions as indicated by asterisks in the structure shown in figure 1.

Two batches with specific activities of 7.84 and 19.4 microcuries/mg were used. The radiopurity was ≥ 93% as determined by high-pressure liquid chromatography (HPLC) and two-dimensional thin-layer chromatography (TLC).

Loxapine (Lederle Laboratories) [2-chloro-11-(4 methyl-1-piperazinyl) dibenz[*b,f*][1,4] oxazepine] and CL 238,985 [9,10-anthracenedicarboxaldehyde, 2,3-dimethyl-,bis(2-imidazolin-2-ylhydrazone) dihydrochloride] were used as internal standards for the gas-liquid chromatography (GLC) and HPLC assays, respectively.

Dosing

Radiolabeled and unlabeled CL 216,942 were combined and dissolved in normal saline or 10% ethanol-saline solutions to obtain the dosing material of desired specific radioactivity and concentration. The drug was administered iv through the tail, cephalic, and femoral veins to the rat, dog, and monkey, respectively, and orally (gavage) to the rat, at doses ranging from 1 to 20 mg/kg as detailed in table 1.

A multiple-dose study in the dog at 6 mg/kg administered at 3-week intervals was also carried out to reproduce the dose levels and regimens used in some of the ongoing clinical trials. Only the last of the three doses administered contained ^{14}C-labeled drug, to compare the tissue deposition pattern to that following single-dose administration.

Sampling and Analysis

Radioactivity in expired CO_2 was measured by counting aliquots of the trapping solution (ethylene

TABLE 1.—Dosing and sampling schedule for CL 216,942

Species	Route	Dose (mg/kg)	Total ^{14}C dose/animal (microcuries)	No. of animals	Sampling time or time animal was killed after dosing	
					Hrs	Days
Rat	Single iv	20	35.9–44.0	44	1–360	
	Single iv (bile cannulated)	20	17.4, 21.3	2	1–6	
	Single oral (bile cannulated)	20	32.4	2	1–6	
	Single iv	10	11–15	14	0.5–264	
Dog	Single iv	10	—	2	0.1–24	
	Single iv	1.25	5.0, 5.5	2	0.1–48	
	Single iv	12.5	4.8	2	0.1–48	
	Single iv	10	171.5–235	3		1,31,71
	Single iv	6	307.1	1		5
	Multiple iv*	6	461	1		5†
Monkey	Single iv	1	37.3, 43.5	2	0.1–24	
	Single iv	10	101.4, 155.2	2	0.1–48‡	7–73§

*Three doses at 3-wk intervals; last dose with ^{14}C-radiolabeled drug.
†After last dose.
‡Sampling.
§Animal killed.

glycol monomethyl ether:ethanolamine, 2:1) at several time intervals after dosing.

Urine and feces were collected at 24-hour intervals. Bile was collected from four rats at 1-hour intervals up to 6 hours after dosing; the bile ducts were cannulated under light ether anesthesia. Blood samples were obtained by heart puncture in the rat and from the jugular and femoral veins in the dog and monkey, respectively.

Tissue deposition of total radioactivity was investigated by whole-body autoradiography (2) (rat and dog) and by scintillation counting of tissue samples in all three species.

All samples were counted in duplicate in a Beckman Model LS 9000 liquid scintillation spectrometer provided with a microprocessor for the calculation of absolute radioactivity. Aliquots of plasma, bile, urine, and tissue homogenate were counted directly in Preblend 3a70B scintillation cocktail (Research Products Co, Elk Grove Village, IL).

Tissue samples, blood samples, and fecal homogenates in 20% ethanol were air-dried in Combusto-Cones (Packard Instrument Corp, Downer's Grove, IL) and combusted to $^{14}CO_2$ in a Packard Tri-Carb sample oxidizer, Model 306.

Drug levels were analyzed by GLC with electron capture detection and/or by HPLC as follows.

GLC Assay

Serum or plasma aliquots (0.5–2 ml), alkalinized to pH 9 with 1 N sodium hydroxide, were extracted with dichloromethane. Loxapine was used as an internal standard. The organic layer was evaporated under nitrogen, and the dry residue was reacted with 150 µl of acetylating reagent (trifluoroacetic anhydride; Pierce Chemical Co, Rockford, IL) for 2 hours at 60°C and dissolved in 100 µl of ethyl acetate. Aliquots of 1–2 µl were injected for analysis.

Chromatographic conditions.—Chromatograph: Tracor Model 222; column: 6 ft, 3% OV17, temperature set at 225°C; detector: ^{63}Ni, temperature set at 290°C; carrier gas: 5% methane, 95% argon at a flow rate of approximately 50 ml/min.

Standard curves for each set of samples were obtained by analyzing control plasma containing known concentrations of CL 216,942. Drug concentrations (in ng/ml) were calculated from peak area ratios by using a Hewlett Packard 3354 Lab Data System. A linear response was obtained between 10 ng and 4 µg/ml at CL 216,942/loxapine ratios ranging from 1/1 to 1/20. The absolute limit of sensitivity was 1 ng/ml. Quantitation was valid for concentrations of 10 and 20 ng(0.025 and 0.05 nmols)/ml with coefficients of variation of 12.4% and 10.6%, respectively.

HPLC Assay

Extraction.—Serum or plasma aliquots (1 ml), containing 5 µg of internal standard, CL 238,985, were extracted with dichloromethane. The pH was made alkaline by adding 100 µl of concentrated NH_4OH. The organic layer was separated and evaporated to dryness under a stream of nitrogen. The residue was dissolved into 150 µl of HPLC mobile phase, and aliquots of 5–50 µl were injected for analysis.

HPLC conditions.—Column: Waters Bondapak C_{18}, 10 µ at 25°C; mobile phase: $CH_3CN:H_2O:HCOONH_4$ sol. pH 4.3 (35:60:5) at 0.4 ml/min; detection: UV at 260 nm.

Results.—Linearity: 10–2000 ng/ml; detection: 1 ng/ml; reproducibility: 10 ng/ml (SD = 7.7%), 20 ng/ml (SD = 6.2%); stability in mobile phase: > 24 hrs at 25°C, > 72 hrs at 4°C; extraction efficiency: > 80%.

Metabolic Profile

Plasma, urine, fecal and tissue homogenates, and bile were analyzed for metabolic profile by TLC and HPLC. For TLC, 0.25-mm Silica Gel 60 F-254 (Merck) and Silica Gel GF (Analtech) plates were used. The solvent systems were toluene: methanol:ammonium hydroxide (100:50:0.5) and chloroform:methanol (3:2) for the two types of plates, respectively. The radioactive areas were located by autoradiography and scraped off the plates; the silica powder was counted directly or extracted for isolation and identification by mass spectrometry.

HPLC was done under the following conditions. High-pressure liquid chromatograph: Micromeritics Model 7000; column: Partisil 10/25 ODS; mobile phase: 0.2 M citric acid:methanol (70:30); flow rate: 1.2 ml/min; temperature: ambient; detector (UV): Micromeritics Model 785; λ = 260 nm.

Pharmacokinetic parameters were calculated by using an interactive graphic software package: IG-PHARM (3).

RESULTS AND DISCUSSION

The excretion pattern of the drug in the dog is summarized in table 2. Total recoveries in the dog were low, averaging < 40% of the administered dose over a period of 96 hours, with most of the radioactivity excreted in the feces in the first 48 hours after dosing. A similar excretion pattern was found in the monkey. As seen in table 3, urine and feces were collected daily for 7 days, and at six additional time intervals up to 36 days. Less than 50% of the administered dose was excreted in the first 7 days of the study. Only the rat, as seen in table 4, excreted most of the administered drug over a period of 10 days (almost 85%), again with a significantly higher percentage found in the feces. The cumula-

TABLE 2.—Excretion of radioactivity by dogs

Time after dose (hrs)	% of dose excreted*					
	1.25-mg/kg dose			12.5-mg/kg dose		
	Urine	Feces	Total	Urine	Feces	Total
0–24	2.79	18.85	21.64	2.70	11.32	14.02
24–48	0.45	7.88	8.33	0.36	9.35	9.71
48–72	0.32	2.36	2.68	0.27	2.97	3.24
72–96	0.22	1.74	1.96	0.12	1.70	1.82
Cumulative total	3.78	30.83	34.61	3.45	25.34	28.79

*Mean values from 2 dogs/dose level.

TABLE 3.—Excretion of radioactivity by monkeys

Time after dose (days)	% of dose excreted*					
	1-mg/kg dose			10-mg/kg dose		
	Urine	Feces	Total	Urine	Feces	Total
1	1.96	4.13	6.09	3.46	10.55	14.01
2	0.73	19.16	19.89	1.16	10.95	12.11
3	0.49	7.83	8.32	0.68	8.64	9.32
4	0.48	1.12	1.60	0.69	2.48	3.17
5	0.34	0.74	1.08	0.64	1.89	2.53
6	0.53	0.94	1.47	0.50	0.92	1.42
7	0.46	0.52	0.98	0.46	2.76	3.22
Cumulative total, Days 1–7	4.99	34.44	39.43	7.59	38.19	45.78
16	0.55	0.45	1.00	0.24	0.44	0.68
19	0.24	0.17	0.41	0.15	0.07	0.22
25	0.20	0.11	0.31	0.14	0.07	0.21
29	0.19	0.20	0.39	0.13	0.08	0.21
32	0.19	0.16	0.35	0.12	0.12	0.24
36	0.17	0.13	0.30	0.11	0.04	0.15

*Mean values from 2 monkeys/dose level.

TABLE 4.—Excretion of radioactivity by rats

Time after dose (days)	% of 10-mg/kg dose*		
	Urine	Feces	Total
1	14.81	23.71	38.52
2	1.84	22.57	24.41
3	0.68	7.13	7.81
4	0.42	3.15	3.57
5	0.28	6.99	7.27
6	0.23	0.46	0.69
7	0.16	0.41	0.57
8	0.10	0.43	0.53
9	0.13	0.76	0.89
10	0.10	0.52	0.62
Cumulative total	18.75	66.13	84.88

*Mean values from 2 rats.

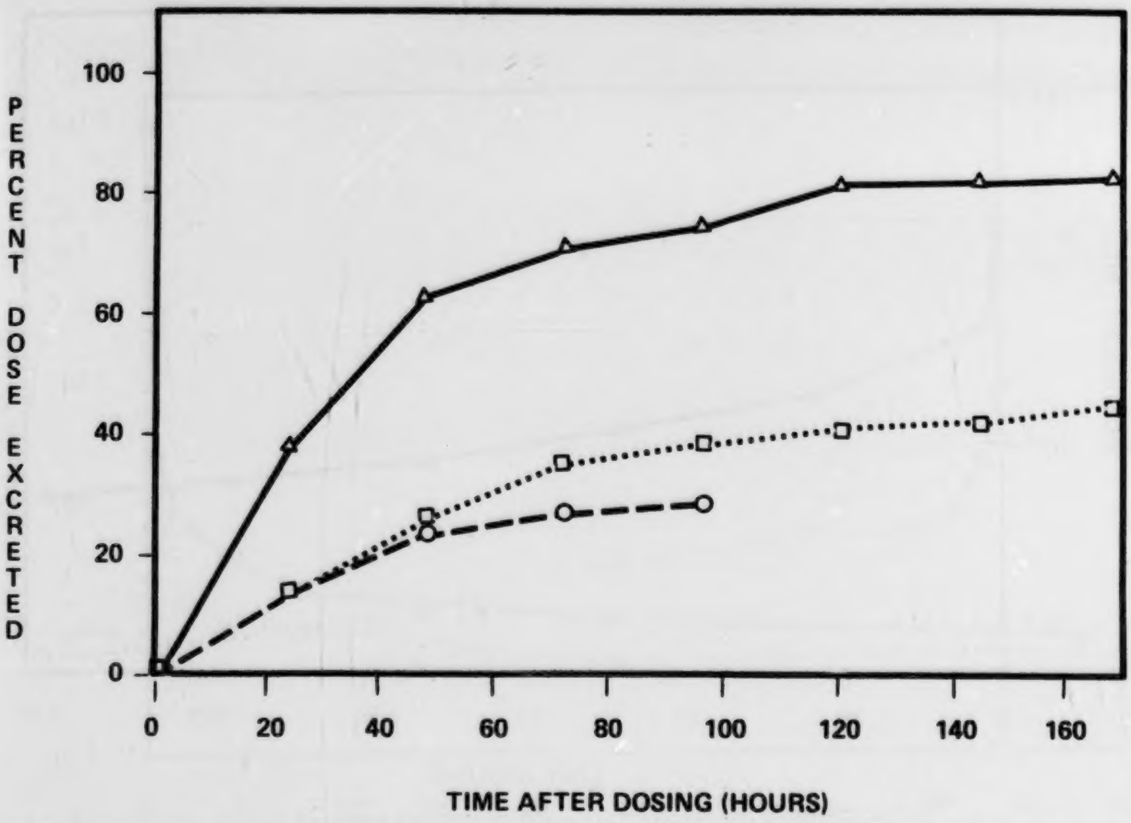

FIGURE 2.—Cumulative excretion of total radioactivity (urine + feces) in the rat (△), monkey (□), and dog (○) following single iv doses of ^{14}C-CL 216,942 (10–12.5 mg/kg).

tive excretion in all three species is shown in figure 2. Each point represents the combined percent of administered dose in both urine and feces.

Whole-blood and plasma concentrations of total radioactivity as a function of time in the dog are shown in figure 3 and indicate much higher drug concentrations in the rbcs than in the plasma. The rbc/plasma drug concentration ratio decreased from about 10/1 at early time intervals to about 2/1 by 24 hours.

The partition of CL 216,942 in dog blood in vitro was studied. Most of the radioactivity (approximately 90%) was found in the rbc fraction, with 56%–60% in the lysate and 33%–34% in the cell membrane fraction. About 9%–11% of the radioactivity was found in the serum or plasma fractions.

The curves in figure 3 also indicate a biphasic disappearance of the drug with relatively long elimination half-lives of about 2–3 days.

A similar pattern was found in the monkey and rat. Figure 4 shows the variation of drug plasma concentrations with time in the monkey as measured by GLC and scintillation counting. Figure 5 shows the plasma concentrations versus time curves in the rat as measured by GLC and HPLC. The elimination half-lives were relatively long, about 2–8 days in all three species. High volumes of distribution of 13, 184, and 1243 liters were calculated for the rat, monkey, and dog, respectively. These data suggested a significant tissue deposition of the drug and correlated with the slow elimination in the excreta of these animals, especially the dog and monkey. Therefore, tissue deposition was investigated in all three species, as described in the Materials and Methods section, at various time intervals.

Tissue distribution in the rat at 1 hour after dosing with 10 mg of ^{14}C-CL 216,942/kg, as evidenced by whole-body autoradiography, is shown in figure

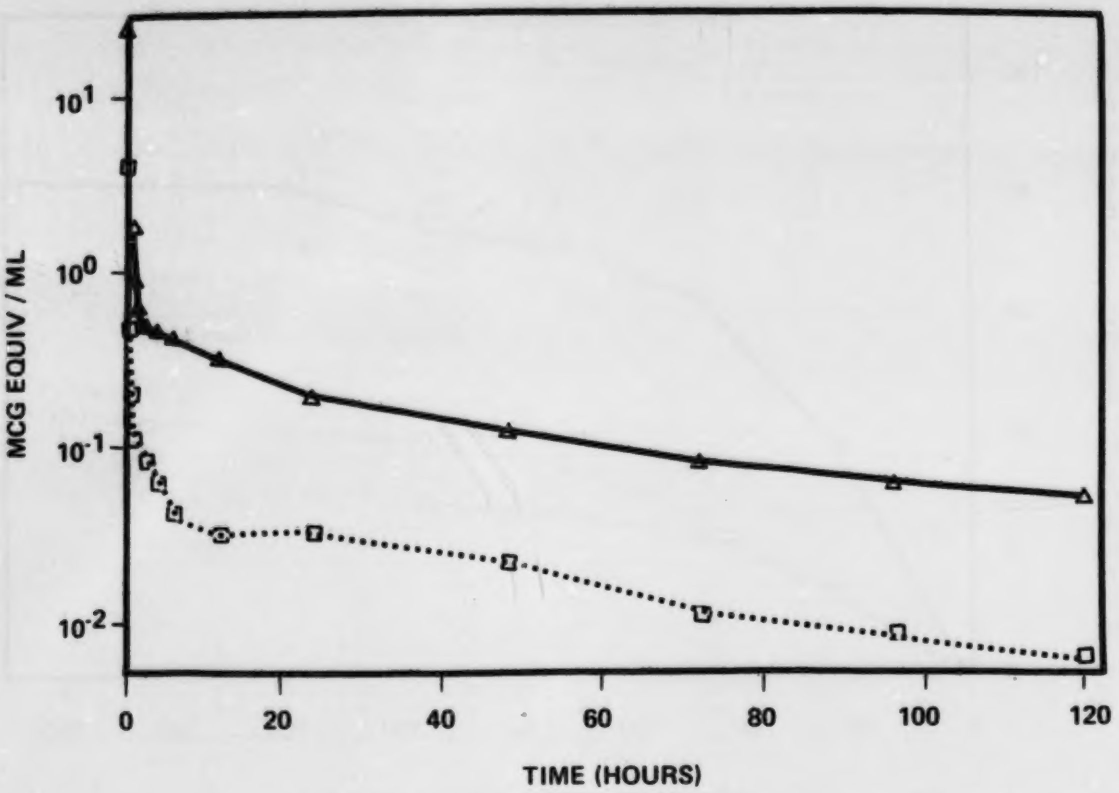

FIGURE 3.—CL 216,942 concentrations in whole blood (△) and plasma (□) in the dog following a single iv dose of 12.5 mg/kg of ^{14}C-radiolabeled drug. Each point is the mean value from 2 animals.

6. The dark areas indicate the organs and tissues containing high concentrations of radioactive drug. Quantitative measurements of radioactivity in rat tissues removed at several time intervals (two rats/time period) indicate significant drug uptake in most of the highly perfused organs: liver, heart, lung, kidney, and spleen; very low, if any, radioactivity was found in the brain, suggesting that the drug does not cross the blood-brain barrier.

Elimination half-lives of the same order of magnitude (48–108 hours) were roughly estimated in some tissues containing high drug concentrations (fig 7).

Tissue distribution of radioactivity in dogs killed at 1, 31, and 71 days after a single iv dose of 10 mg/kg and 5 days after single and multiple dosing at 6 mg/kg is detailed in table 5. The amount of drug in the tissues decreased from about 28% at 24 hours to about 10% of the administered dose over 71 days after dosing. The highly perfused organs, ie, heart, lung, liver, kidney (cortex), spleen, pancreas, gall bladder, pituitary, and salivary glands, contained the highest drug concentrations. Very low radioactivity, if any, was found in the brain and spinal cord.

A similar tissue distribution pattern was seen in the monkey at 7 and 73 days after the administration of single iv doses of 10 mg/kg (table 6).

To investigate the tissue deposition of ^{14}C-CL 216,942 in subcellular fractions, rat liver homogenates were treated with trichloroacetic acid and extracted with various solvents as shown in figure 8. About 75% of radioactivity (^{14}C-CL 216,942) was found in the lipid solvent extracts and about 25% was found in the protein and nucleic acid precipitates. About 2% of the radioactivity contained in the liver was found in the nucleic acid-enriched fraction isolated by boiling the precipitate with 10% NaCl.

In vitro studies on DNA-drug interaction were done by equilibrium dialysis with calf thymus and phage lambda DNA (to be published separately).

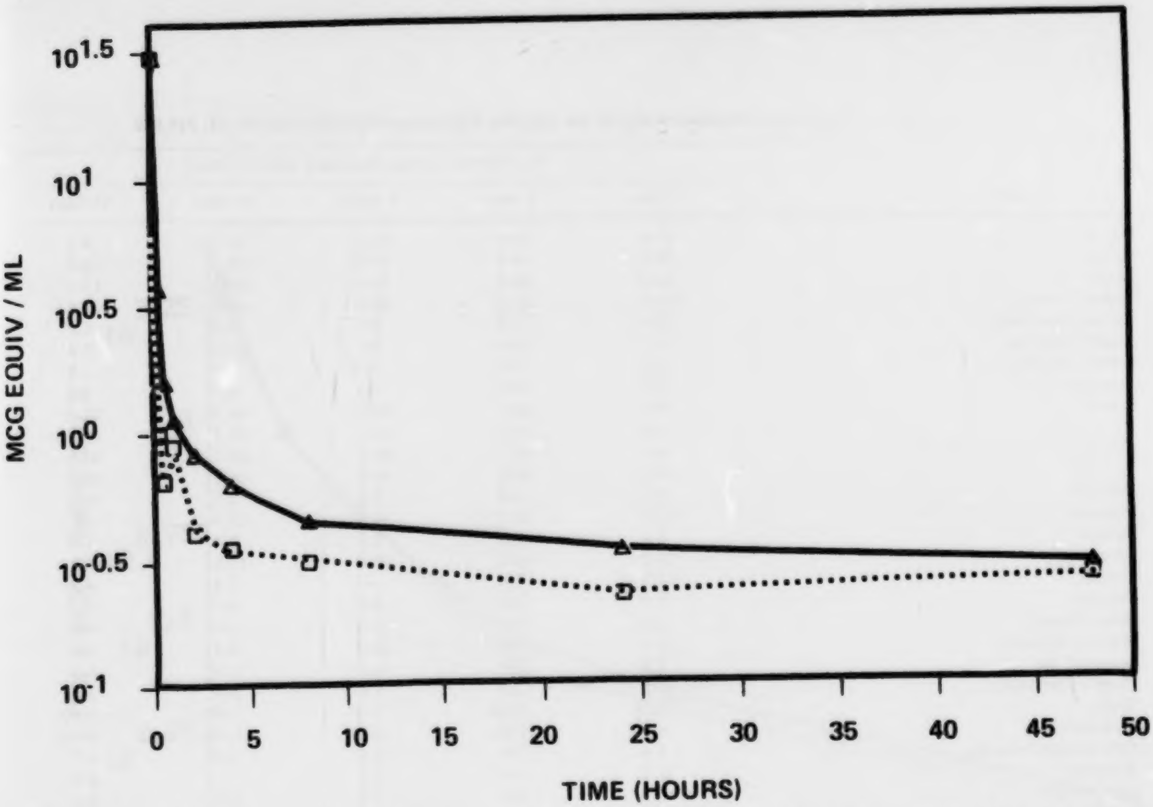

FIGURE 4.—Plasma concentrations of ^{14}C-CL 216,942 in the monkey as measured by scintillation counting (△) and GLC (□) following a single iv dose of 10 mg/kg. mcg equiv. = microgram of total drug-related compounds as measured by scintillation counting and/or GLC. (Metabolism studies indicate that most of the radioactivity [> 90%] is unchanged parent drug.)

Preliminary data indicate a significant drug concentration-dependent binding to DNA of up to 90%. The binding was not significantly changed at various ionic strengths (0.02–0.3 M). The data suggest that intercalation of the drug into the DNA molecule may be the predominant type of CL 216,942-DNA interaction. The binding affinity constant and the number of binding sites were 7.4×10^{5} and 0.31, respectively, at DNA concentrations of 0.5×10^{-5} M in the range of 0.4 to 4×10^{-5} M drug concentrations.

The metabolic profile was investigated by TLC and HPLC in plasma, urine, bile, feces, and several tissues as described in the Materials and Methods section. The main radioactive compound, accounting for approximately 90% of total radioactivity extracted from these samples, was found to be unchanged CL 216,942 by direct-probe electron impact mass spectrometry. The extraction efficiency was about 90%–95%, except for fecal and liver homogenates from which only 70% of total radioactivity was extractable. Therefore, bile collected from rats with cannulated bile ducts was examined directly, without extraction, by TLC. There was only one radioactive spot having the same R_f value as authentic ^{14}C-CL 216,942, accounting for 94% of the applied radioactivity.

When unextracted 0–24-hour urine samples from a dog receiving 12.5 mg/kg of ^{14}C-CL 216,942 were analyzed by HPLC, a compound more polar than CL 216,942,accounting for about 6% of total radioactivity, was isolated in addition to the unchanged

TABLE 5.—Tissue distribution of radioactivity in the dog following iv administration of ^{14}C-CL 216,942

Tissues	CL 216,942 μg equivalents/g after dosing*				
	1 day	5 days	5 days†	31 days	71 days
Heart	31.8	12.5	10.4	15.7	9.1
Lung	39.9	19.0	19.5	14.2	14.3
Liver	54.4	25.9	15.5	16.3	17.1
Kidney (cortex)	181.0	41.3	38.2	48.7	41.5
Kidney (medulla)	17.1	1.8	2.6	1.0	1.3
Small intestine	11.1	2.8	2.8	4.5	2.4
Large intestine	6.0	2.0	2.2	2.6	1.2
Stomach	12.0	6.0	6.4	4.9	3.6
Spleen	46.2	29.6	32.5	28.3	23.2
Pancreas	17.4	15.6	12.1	30.7	18.5
Salivary or parotid glands	24.1	24.7	20.5	21.6	20.5
Thyroid	24.8	25.4	14.3	34.7	18.3
Thymus	4.7	3.2	2.0	7.7	2.8
Prostate	27.7	15.1	14.1	13.4	6.1
Testes	0.8	0.7	1.1	1.4	1.3
Pituitary	19.2	22.2	12.6	17.5	12.1
Tonsils	11.0	7.4	—	7.4	4.2
Tongue	7.9	4.6	4.0	5.3	3.3
Bone (femur)	0.6	0.7	0.6	0.4	0.3
Marrow (femur)	5.4	7.0	12.9	5.4	3.0
Omental fat	2.9	1.0	0.5	2.4	0.7
Muscle (femoral)	5.3	2.5	1.6	3.7	1.9
Brain	0.08	0.03	0.03	0.06	0.03
Eye (lens)	0.01	0.01	0.01	0.04	0.05
Eye (cornea muscle)	3.0	1.8	1.5	4.8	5.7
Adrenal	11.6	8.2	8.2	7.1	3.6
Epididymis	3.2	2.6	1.8	3.6	2.4
Lymph node (mesenteric)	15.1	10.1	7.7	13.4	6.2
Lymph node (popliteal)	—	9.0	152.0	11.8	5.0
Diaphragm	8.6	4.3	3.7	4.7	2.2
Bladder	4.1	2.2	2.3	2.1	—
Gall bladder	48.5	16.8	17.9	9.7	7.8
Spinal cord	0.04	0.04	0.07	0.07	0.05
Trachea	1.9	1.0	0.7	1.1	0.7
Bile (μg/ml)	11.9	10.8	5.1	—	3.4
Aorta	2.3	1.2	1.5	0.5	0.7
Sciatic nerve	2.1	0.6	0.8	1.5	0.5
Skin	—	—	—	2.6	—
Esophagus	—	3.8	2.5	3.3	2.0
Plasma and/or blood (μg/ml)	<0.1	<0.1	—	—	<0.1

*Each value is the mean from 3 tissue samples. — = not determined.

†After multiple dose (*see* table 1).

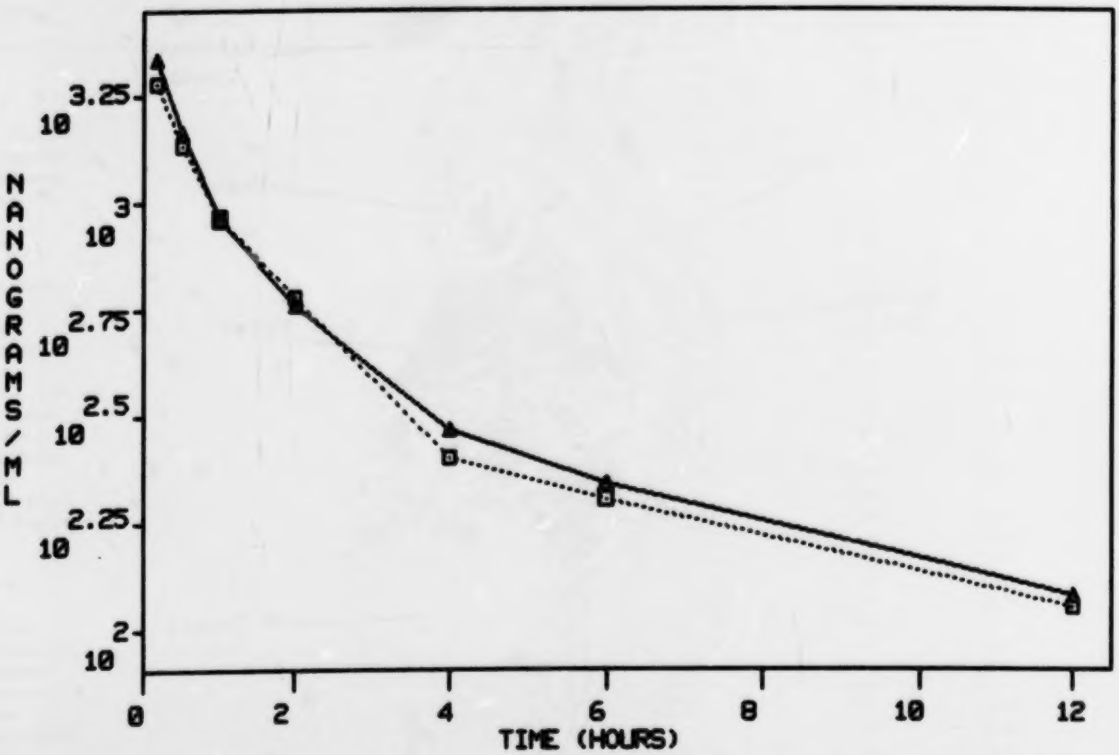

Figure 5.—CL 216,942 concentrations in rat plasma following a single iv dose of 20 mg/kg, as measured by HPLC (△) and GLC (□).

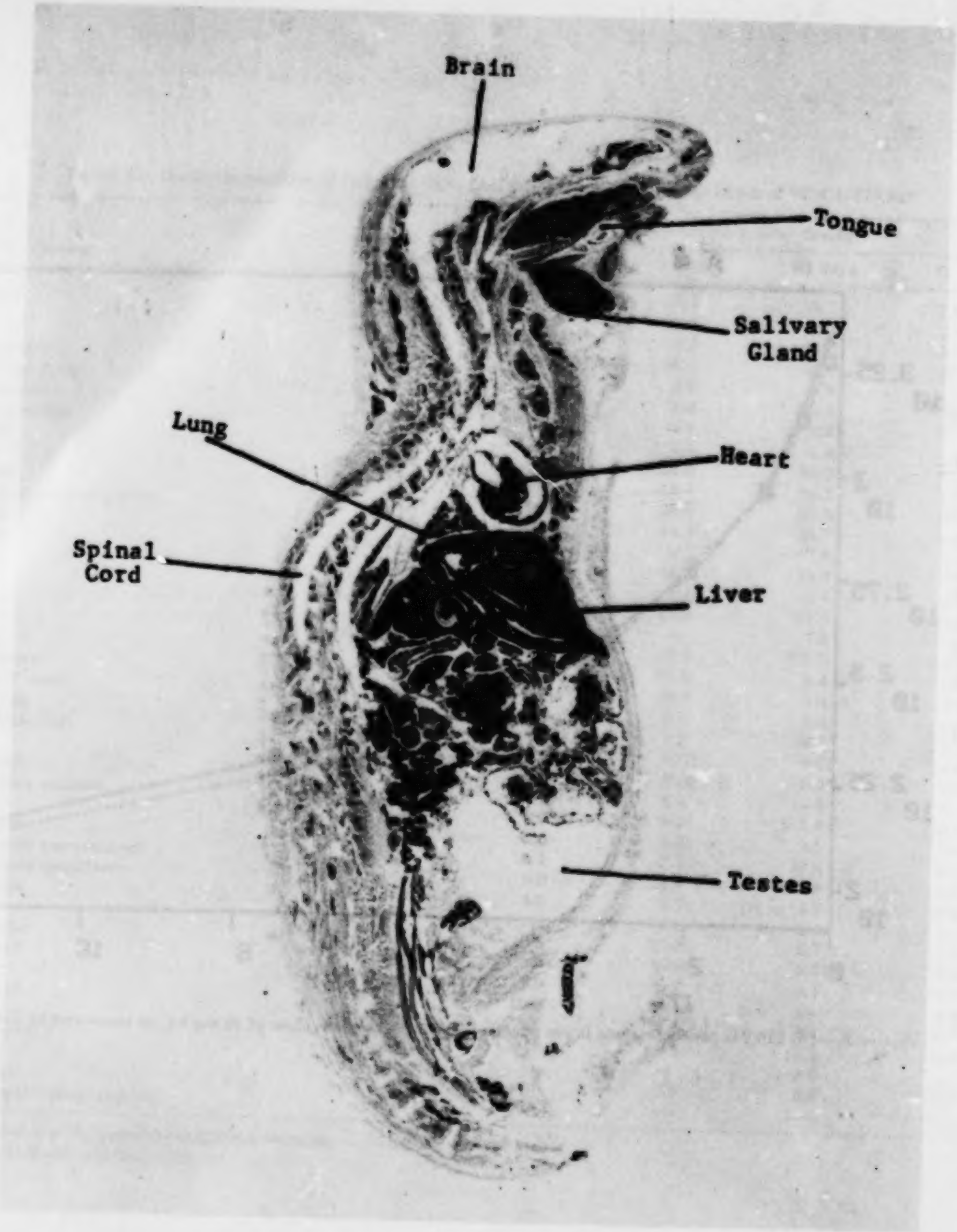
Brain
Tongue
Salivary Gland
Lung
Heart
Spinal Cord
Liver
Testes

FIGURE 6.—Whole-body autoradiography of ^{14}C-CL 216,942 in the rat at 1 hr after an iv dose of 20 mg/kg.

TABLE 6.—Tissue distribution of radioactivity in the monkey following iv administration of 10 mg/kg of ^{14}C-CL 216,942

Tissue	Distribution after dosing*			
	7 days		73 days	
	μg equivalent/g	% of dose†	μg equivalent/g	% of dose†
Heart	45.88	1.23	9.52	0.26
Lung	7.56	0.61	1.93	0.08
Liver	29.54	6.90	6.28	1.16
Kidney (cortex)	86.50	3.47	12.21	0.42
Small intestine	5.14	0.90	0.95	0.11
Large intestine	4.36	0.95	0.87	0.08
Pancreas	55.80	0.98	14.20	0.17
Spleen	28.50	1.78	4.61	0.07
Stomach	13.16	0.94	4.05	0.15
Uterus	9.6	0.20		
Marrow (femur)	14.1		2.70	
Fat‡,§	1.86	3.53	1.26	2.31
Muscle§	2.00	8.60	1.22	5.00
Salivary glands	19.82	0.24	28.14	0.16
Thyroid	32.68	0.04	9.95	0.02
Thymus	11.79	0.01	3.41	0.02
Lymph nodes	25.04		3.45	
Diaphragm	8.06	0.15	2.08	0.01
Parotid glands	16.30	0.30	—	—
Adrenals	22.13	0.05	5.82	0.01
Plasma (μg/ml)	0.08		< 0.05	
Urinary bladder	3.15	0.04	0.58	0.01
Testes			1.50	0.06
Eyes	2.96	0.05	0.82	0.01
Femur	—	—	0.19	0.01
Brain	0.03	< 0.01	< 0.05	< 0.01
Spinal cord	0.03		0.05	
Epididymis	—	—	1.96	0.01
Gall bladder	16.01	0.02	3.06	< 0.01
Sciatic nerve	2.76		0.46	
Prostate			3.70	0.02
Seminal vesicles			3.84	0.03
Pituitary	50.5	0.01	14.10	< 0.01
Ovaries	2.18	< 0.01		
Marrow (sternum)	11.53			
Tongue	7.52	0.32		

*— = not determined.

†In whole organ or tissue.

‡Average of omental, epididymal, and/or subcutaneous fat.

§Total weight estimated from Biochemists Handbook (Long C, ed), 1961, p 639.

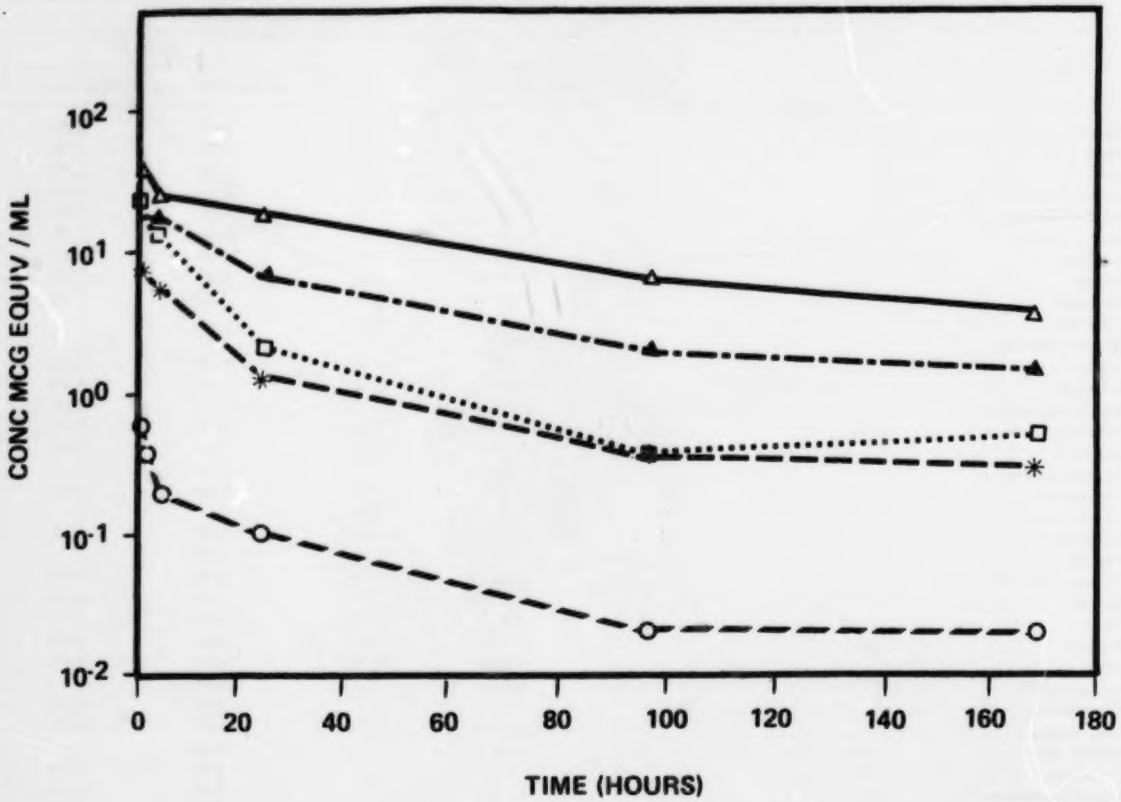

FIGURE 7.—Concentrations of total radioactive drug in rat tissue after single iv doses of 10 mg/kg of CL 216,942. Each point is the mean value from 2 rats killed/time period. Tissues: spleen (△), pancreas (□), adrenal (▲), bladder (*), and plasma (○).

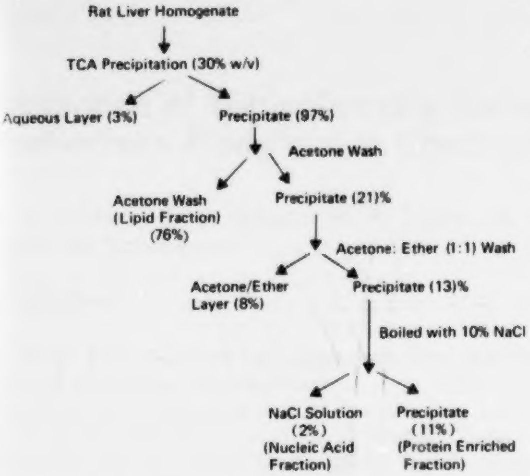
Rat Liver Homogenate
TCA Precipitation (30% w/v)
Aqueous Layer (3%)
Precipitate (97%)
Acetone Wash
Acetone Wash
(Lipid Fraction)
(76%)
Precipitate (21)%
Acetone: Ether (1:1) Wash
Acetone/Ether
Layer (8%)
Precipitate (13)%
Boiled with 10% NaCl
NaCl Solution
(2%)
(Nucleic Acid
Fraction)
Precipitate
(11%)
(Protein Enriched
Fraction)

FIGURE 8.—Fractionation of cellular CL 216,942 by solvent extraction (% of total radioactivity). TCA = trichloroacetic acid.

parent drug. No other significant biotransformation of the drug was observed.

To investigate the oral absorption of CL 216,942, the drug was administered both by gavage and iv to rats with cannulated bile ducts, and drug excretion in the bile was measured. About 15% of the administered dose was found in the bile collected up to 6 hours from rats dosed iv; < 0.5% was found in the bile of those receiving the oral dose, indicating very poor, if any, drug absorption.

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Response of Transplantable Tumors of Mice to Anthracenedione Derivatives Alone and in Combination With Clinically Useful Agents[1,2]

T. H. Corbett,* B. J. Roberts, M. W. Trader, W. R. Laster, Jr, D. P. Griswold, Jr, and F. M. Schabel, Jr[3]

SUMMARY

Other investigators have demonstrated that dihydroxyanthracenedione (DiOHA) and anthracenedione acetate (AA) are active against a broad spectrum of transplantable mouse tumors. DiOHA and AA are in clinical trial in the US; AA is in clinical trial in Europe. Because of the broad spectrum of activity of these DNA binders against murine tumors and due to their promising clinical utility, we have evaluated these agents in combination with a variety of clinically useful antitumor drugs. Studies were carried out against three colon adenocarcinomas (38, 06/A, and 11/A), three mammary adenocarcinomas (13/C, 16/C, and 14), and two lymphocytic leukemias (P388 and L1210). The therapeutic synergism of one of these combinations, DiOHA and cisplatin, has been previously reported. Four additional combinations which were found to have confirmed therapeutic synergism are reported here: DiOHA and palmO-ara-C, DiOHA or AA and 5-FU, DiOHA or AA and vincristine, and DiOHA and dacarbazine. The combination toxicity indices (CTI; a measure of the degree of overlap in dose-limiting toxic effects) were obtained for all the following combinations: DiOHA and palmO-ara-C = 1.25–1.6; DiOHA or AA and 5-FU = 1.2–1.3; DiOHA or AA and vincristine = 1.6; and DiOHA and dacarbazine = 1.3–1.5. A CTI of 1.0 indicates complete overlap in dose-limiting toxic effects, eg, only 50% of the maximum tolerated dose of each agent can be used in combination. On the other hand, a CTI of 2.0 indicates no overlap in toxicity, and 100% of the maximum tolerated dose of each agent can be used in combination.

[Cancer Treat Rep 66:1187–1200, 1982]

Anthracenedione and dihydroxyanthracenedione (DiOHA) have demonstrated antitumor activity against a wide spectrum of transplantable animal tumors (1,2).[4] The antitumor activity of the parent compound, anthracenedione, was discovered through random screening, and later its diacetate salt, anthracenedione acetate (AA), was synthesized in an effort to improve water solubility. The 1,4-dihydroxy congener and its dihydrochloride (DiOHA) were selected from a number of analogs because of greater antitumor activity against certain transplantable tumors and because of greater potency

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[1] Received July 10, 1981; revised Sept 30, 1981; accepted Nov 12, 1981.

[2] Supported by contract N01-CM-97309 (Division of Cancer Treatment) and grants CA-26485 and CA-17303 (National Large Bowel Cancer Project, the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] We gratefully acknowledge the technical assistance of G. Gamble, J. Seay, C. Howard, P. Blair, R. Apperson, S. Terry, T. Ayer, M. Witt, J. McKinstry, B. McDonough, M. Jackson, and P. Holum in the collection of laboratory data and the assistance of T. Ayer, C. Schulman, and L. Muglach in manuscript preparation.

[4] Corbett TH, Griswold DP, Jr, and Schabel FM, Jr. Unpublished results.

**Reprint requests to:* T. H. Corbett, PhD, Southern Research Institute, PO Box 3307-A, 2000 9th Ave S, Birmingham, AL 35255.

(only one tenth of the dose was required to reach equivalent toxicity in mice) (1,2). These agents are similar in action to doxorubicin, either binding to or intercalating with DNA (DNA binders) (1). Leukopenia was the major dose-limiting toxic effect observed in clinical trials with AA (European trial) or DiOHA (US trial) (3).

Because of the broad spectrum of activity against mouse tumors and because of their promising clinical utility, we have evaluated these agents in combination with a variety of clinically useful antitumor drugs. The therapeutic synergism (TS) of one of these combinations, DiOHA and cisplatin, has been previously reported (4). The results of five additional combinations are reported herein.

MATERIALS AND METHODS

Studies were carried out against three colon adenocarcinomas (38, 06/A, and 11/A), three mammary adenocarcinomas (13/C, 16/C, and 14), and two lymphocytic leukemias (P388 and L1210). Tumors were maintained in the mouse strain of origin and were transplanted into the appropriate F_1 hybrid for chemotherapy trials. Individual body weights for all mice within each experiment were 18–22 g at the time of tumor implant. The mice were supplied food (Wayne-Lab Blox F-6, which contained 6% fat) and water ad libitum. The mice and antitumor agents were obtained through the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

Chemotherapy for the solid tumors was started within 1–5 days of solid tumor implantation when the number of tumor cells was relatively small (30–60 mg, early-stage disease), or, alternatively, chemotherapy was delayed until the tumors became relatively large (~ 200 mg, advanced-stage disease). A 200-mg tumor contains approximately 2×10^8 total cells. Ten normal mice (with no tumors) were often matched to each tumor-bearing treatment group and treated with the same agents at the same doses, schedules, days of administration, and drug route. In this way, drug-induced toxicity (lethal toxic effects and weight loss) could be clearly separated from the effects of the tumors.

Treatment of mice with leukemia was initiated on the sixth day after intraperitoneal implant of 10^6 P388 cells or on the second day after intravenous implant of 10^7 L1210 cells, at which time either tumor cell population had proliferated to about 2×10^8 cells.

The animals necessary to begin an experiment were pooled and the nontumor toxicity controls were then removed. The remaining mice were implanted with the tumors and again pooled before unselected distribution to the various treatment groups (early-stage tumor treatment).

If the experiment was designed for advanced-stage treatment, the solid tumors were allowed to grow to the desired range (animals with tumors not in the desired range were excluded). The mice were then pooled and unselectively distributed to the various treatment and control groups. Tumors were measured with a caliper twice weekly until the animal died. Solid tumor weights were calculated from two-dimensional measurements:

$$\text{Tumor weight (mg)} = \frac{a \times b^2}{2},$$

where a is the length (mm) and b is the width (mm). Each mouse was weighed at 7-day intervals, beginning the first day of drug treatment. The mice were checked daily for deaths.

The following quantitative end points were used to assess antitumor effectiveness.

1. Percent increase in host lifespan (% ILS) = $\frac{T-C}{C} \times 100$, where T = treated and C = control median days of death. Tumor-free survivors were excluded from these calculations.
2. Tumor growth delay (T-C), where T = the median time (days) required for the treatment-group tumors and C = the control-group tumors (median of ten tumors each) to reach a predetermined size (usually 750 or 1250 mg). Tumor-free survivors were excluded from these calculations. In our judgment, this value is the single most important criterion of antitumor effectiveness because it allows the quantitation of tumor cell kill. It can be considered similar to the "duration of remission" value used clinically.
3. Regressions: advanced-stage primary site tumor.
   Complete regression (CR) = regression below the limit of palpation.
   Partial regression (PR) = > 50% reduction in tumor (CRs were included in the PRs).

Quantitation of Tumor Cell Kill

For subcutaneously growing tumors, the $\log_{10}$ cell kill/dose was calculated from the following formula:

$$\text{Log}_{10}\ \text{kill/dose} = \frac{\text{T-C value in days}}{(3.32)\ (\text{Td})\ (\text{No. of doses})},$$

where T-C is the tumor growth delay as described above and Td is the tumor volume doubling time in days measured from a best fit straight line of the control-group tumors in exponential growth (range, 100–400 mg).

In those cases in which survival was the only end point of therapeutic effectiveness (leukemias and intravenously implanted tumor cells), the T-C value was the survival time in days (tumor-free survivors excluded) for the treated group (T) minus the survival time in days for the control group (C). The Td values were obtained from a plot of the median days of death (tumor-free survivors excluded) against $\log_{10}$ dilutions of the tumor cells in untreated mice.

The $\log_{10}$ cell kill (gross) was the $\log_{10}$ kill/dose multiplied by the number of doses, or $\frac{\text{T-C in days}}{(3.32)(\text{Td})}$.

$$\text{Log}_{10}\text{ cell kill (net)} = \frac{(\text{T-C value in days}) - (\text{duration of treatment in days})}{(3.32)(\text{Td})}.$$

If the $\log_{10}$ cell kill (net) value was positive, there were fewer cells present at the end of therapy than at the start. If, on the other hand, the value was negative, the tumor grew under treatment. A positive gross value with a negative net value indicated inhibition of growth of the tumor cell population during drug treatment.

Determination of TS

The methods used for the determination of TS have been described in detail elsewhere (5). Briefly, a combination is considered to have TS if it is therapeutically superior to the optimum use of either agent alone (6,7). In all trials listed, comparisons between the single agents and the combination were made at the maximum tolerated dose ($\leq$ LD10, except in one case in which an LD20 was used). Doses approximately 1.5 times those listed were excessively toxic within the same experiment, inducing either $>$ 4 g of weight loss/mouse ($\sim$ 20% of body weight) or death in $\geq$ 20% of the mice.

RESULTS

The biologic characteristics of the mouse tumor models are listed in table 1. The model tumor used to evaluate each drug combination was responsive to each of the agents used singly. It has been our experience that TS is unlikely to occur with cytotoxic agents when the tumor is unresponsive or only marginally responsive to one of the agents in the combination (8–10). The combinations described were evaluated only against those tumors for which data are presented; ie, no negative or positive results are available other than those presented.

Four anthracenedione combinations were found to have TS in two or more experiments. The DiOHA and palmO-ara-C combination was found to have marked TS against colon adencarcinoma 06/A, as evidenced by a marked improvement in the number of animals cured in the combination groups as well as an increase in T-C, %ILS, and frequency of regression in those not cured (fig 1, table 2). Similarly, this combination had marked TS against P388 and L1210 leukemias (fig 2, table 2). There were five lower doses of the combination (in various ratios not shown) that produced seven or more cures against P388 leukemia (bearing $> 10^8$ clonogenic cells/mouse at first treatment). No cures were obtained with either of the agents used alone against this tumor.

The combination toxicity index (CTI) (11) values for the four experiments are listed in table 2 and range from 1.25 to 1.6. A CTI of 1.0 indicates that only 50% of the LD10 of each agent can be used in combination without incurring additional toxicity (or alternately with other ratios of the LD10 values of each agent, eg, 70:30, 30:70, 40:60, 60:40, etc). A CTI of 2.0 indicates that 100% of the LD10 dose of each single agent can be used in combination (4,11). The steps used to determine the CTI value of Experiment 1672P1 are shown as a representative of the general method (fig 3, A and B; table 3). The toxicity of each single agent was first plotted as a log-probit graph and the LD10 values determined from a best-fit line of the data (fig 3A). These values were then used to calculate the fractions of the LD10s used in each combination group (table 3). The sum of the fractions of the LD10s was then plotted on the abscissa and the observed lethality on the ordinate to determine the CTI value (fig 3B).

The combination of DiOHA and palmO-ara-C was also attractive because of the lack of cross-resistance. A P388 line resistant to palmO-ara-C (lacking deoxycytidine kinase[5]) was as sensitive to DiOHA as the parent P388/O tumor.[6]

The 5-FU and AA combination was found to have modest TS against colon adenocarcinoma 38 (table 2), and the DiOHA and 5-FU combination was found to have TS against mammary adenocarcinoma 14 (fig 4, table 2). An improvement in T-C, %ILS, and frequency of regression was evident in the combination groups of both experiments (fig 4, table 2). The CTI values for these combinations were 1.25–1.3 (table 2), which means that 60%–65% of the full

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[5] Brockman RW. Southern Research Institute. Personal communication.

[6] Trader MW, and Schabel FM, Jr. Unpublished results.

TABLE 1.—Biologic characteristics of 8 transplantable mouse tumor models

Tumor (transplant generation)	Host of origin	Tumor histology	Grade (degree of differentiation)	Metastasis		Time (days) required for tumors to grow to 500 mg from a trocar implant (range)	Take rate (%) of 30 mg trocar implant	Median Day of death from a trocar implant (range)	Approximate overall doubling time (days) for transplant generation specified (range)
				From ip implant	From sc implant				
Colon 38 (53)	C57BL/6	Colon adenocarcinoma	III	To liver	< 30% to lungs	15–24	> 95	29–51	2.7–4.7
Colon 06/A (10–15)	BALB/c	Colon adenocarcinoma	III	To liver	< 25% to lungs	13–20	> 98*	25–72	2.0–3.4
Colon 11/A (9–12)	BALB/c	Colon adenocarcinoma	III	No data	< 3% to lungs	18–31	> 98*	35–85	2.6–4.0
Mammary 13/C (52)	C3H/He	Mammary adenocarcinoma	III	No data	< 40% to lungs	14–18	> 95	40–49	1.5–2.8
Mammary 16/C (38)	C3H/He	Mammary adenocarcinoma	III	No data	> 80% to lungs	9–15	> 99	29–42	1.6–2.4
Mammary 14 (7–20)	C3H/He	Mammary adenocarcinoma	III	No data	< 25% to lungs	13.5–30	> 98	26.5–50	1.9–2.6
P388† (> 100)	DBA/2	Leukemia	IV	Very high to spleen, marrow, liver, and brain	—	—	100‡	10.5–12†	0.43–0.64
L1210§ (> 100)	DBA/2	Leukemia	IV	Very high to spleen, marrow, liver, and brain	—	—	100¶	4.0§	0.36

*2, 30-mg tumor fragments were trocared at implantation.

†P388 leukemia characteristics based on experiments carried out using 10^6 cells implanted ip.

‡10^2 cells implanted ip.

§L1210 leukemia characteristics based on experiments carried out using 10^7 cells implanted iv.

¶10^2 cells implanted iv.

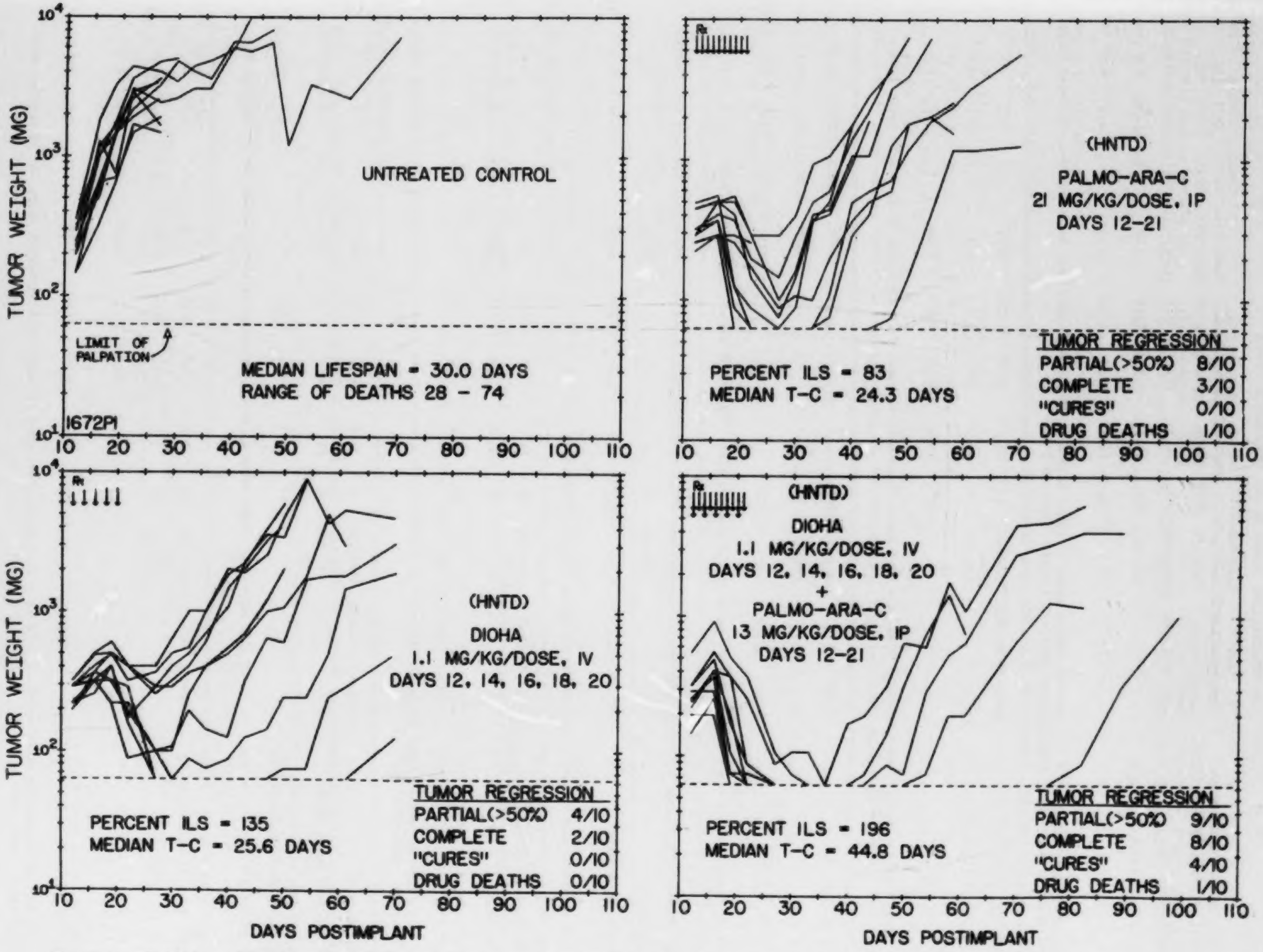

FIGURE 1.—DiOHA and palmO-ara-C treatment of subcutaneously implanted colon adenocarcinoma 06/A in male CDF$_1$ mice. Tumors were 100–400 mg at the time of 1st treatment. HNTD = highest nontoxic dose.

TABLE 2
ANTHRACENEDIONE (DIOHA OR AA) COMBINATION TRIALS

Highest Nontoxic Drug or Combination, Dosage (mg/kg/dose), Route, Schedule (Days)	Tumor (Implantation Site)	Stage of Tumor at First Rx	%ILS	T-C Value (Days)	Regressions Partial (>50%)	Regressions Complete	Tumor-Free Survivors	Log_{10} Kill/Dose	Log_{10} Cell Kill Gross	Log_{10} Cell Kill Net	Combination Toxicity Inde
PalmO-ara-C, 21, ip, qd 12-21	Colon 06/A (SC)	Advanced (100-400 mg)	83	24.3	8/10	3/10	0/10	0.2	2.2	1.4	-
DIOHA, 1.1, iv, 12,14,16,18,20			135	25.6	4/10	2/10	0/10	0.5	2.4	1.6	-
PalmO-ara-C, 13, ip, qd 12-21 DIOHA, 1.1, iv, 12,14,16,18,20			196	44.8	9/10	8/10	4/10	-	4.1	3.3	1.2
Control: Median Day of Death (MDD) = 30.0; time for median tumor to reach 1250 mg = 18.0 days; there were no tumor-free survivors among the 20 control mice. Tumor volume doubling time (Td) = 3.3 days. Expt. 1672 P1.											
PalmO-ara-C, 16.5, ip, qd 11-23	Colon 06/A (SC)	Advanced (100-400 mg)	43	25.0	5/10	4/10	0/10	0.2	3.1	1.6	-
DIOHA, 1.1, iv, 11,13,15,17,19,21,23			13	29.7	1/8	1/8	1/8	0.5	3.7	2.2	-
PalmO-ara-C, 10.5, ip, qd 11-23 DIOHA, 0.67, iv, 11,13,15,17,19,21,23			UE[a]	72.9	8/10	8/10	7/10	-	9.2	7.6	1.3
Control: MDD = 55.0; time for median tumor to reach 1250 mg = 17.5 days; there were no tumor-free survivors among the 20 control mice. Td = 2.4 days. Expt. 1678 F1.											
PalmO-ara-C, 72, ip, 2,6,10	L1210 (IV)	Advanced (10^7 cells)	250	-	-	-	0/10	2.8	8.4	1.7	-
DIOHA, 6.0, ip, 2,6,10			75	-	-	-	0/10	0.8	2.5	-4.2	-
PalmO-ara-C, 48, ip, 2,6,10 DIOHA, 2.7, ip, 2,6,10			400	-	-	-	0/10	-	13.3	6.7	Scant Data
Control: MDD = 4.0; there were no tumor-free survivors among the 20 control mice. Td = 0.36 days. Expt. 1657 CC-635.											
PalmO-ara-C, 72, ip, 6,10,14	P388 (IP)	Advanced (10^6 cells)	108	-	-	-	0/10	3.0	9.1	3.5	-
DIOHA, 4.0, ip, 6,10,14			75	-	-	-	0/10	2.1	6.3	0.7	-
PalmO-ara-C, 48, ip, 6,10,14 DIOHA, 4.0, ip, 6,10,14			183	-	-	-	9/10	-	15.4	9.8	~1.6
Control: MDD = 12.0; there were no tumor-free survivors among the 20 control mice. Td = 0.43 days. Expt. 1657 PS-CC-139.											
5-FU, 41, iv, 16,20,24,28,32	Colon 38 (SC)	Advanced (100-400 mg)	69	18.1	0/10	0/10	0/10	0.4	1.8	0.2	-
AA, 29, iv, 16,20,24,28,32			36	20.8	0/10	0/10	0/10	0.4	2.0	0.5	-
5-FU, 41, iv, 16,20,24,28,32 AA, 18, iv, 16,20,24,28,32			80	31.4	4/10	1/10	0/10	-	3.1	1.5	1.3
Control: MDD = 46.0; time for median tumor to reach 1250 mg = 24.4 days; there were no tumor-free survivors among the 10 control mice. Td = 3.1 days. Expt. 1625 C1.											
5-FU, 62, ip, 24,28,32,36	Mammary 14 (SC)	Advanced (100-400 mg)	41	32.9	9/10	8/10	0/10	0.6	2.4	1.5	-
DIOHA, 0.9, iv, 24,26,28,30,32,34,36			-4	11.7	4/10	1/10	0/10	0.1	0.8	-0.02	-
5-FU, 41, ip,24,28,32,36 DIOHA, 0.6, iv, 24,26,28,30,32,34,36			62	48.3	10/10	10/10	0/10	-	3.6	2.7	1.25
Control: MDD = 77.5; time for median tumor to reach 1250 mg = 41.5 days; there were no tumor-free survivors among the 20 control mice. Td = 4.1 days. Expt. 1712 E1.											
Vincristine, 0.56, iv, 12,14,16,18,20,22	Mammary 13/C (SC)	Advanced (100-400 mg)	29	11.5	1/10	0/10	0/10	0.3	1.7	0.2	-
AA, 20, iv, 12,14,16,18,20,22			24	1.1	0/10	0/10	0/10	0.03	0.2	-1.3	-
Vincristine, 0.35, iv, 12,14,16,18,20,22 AA, 20, iv, 12,14,16,18,20,22			51	19.7	3/10	1/10	0/10	-	2.9	1.5	-
Vincristine, 0.56, iv, 12,14,16,18,20,22 AA, 13, iv, 12,14,16,18,20,22			42	21.2	4/10	4/10	0/10	-	3.2	1.7	1.6
Control: MDD = 35.0; time for median tumor to reach 1250 mg = 16.8 days; there were no tumor-free survivors among the 20 control mice. Td = 2.0 days. Expt. 1628 D1.											

a. Slightly toxic (LD_{20}); %ILS unevaluable due to the large number of cures.

TABLE 2 (Continued)
ANTHRACENEDIONE (DiOHA OR AA) COMBINATION TRIALS

Hi est Nontoxic Drug or Combination, Dosag (mg/kg/dose), Route, Schedule (Days)	Tumor (Implantation Site)	Stage of Tumor at First Rx	%ILS	T-C Value (Days)	Regressions Partial (>50%)	Regressions Complete	Tumor-Free Survivors	Log_{10} Kill/Dose	Log_{10} Cell Kill Gross	Log_{10} Cell Kill Net	Combination Toxicity Index
Vincr tine, 0.5, ip, qd 6-14	P388 (IP)	Advanced (10^6 cells)	14	-	-	-	0/10	0.1	0.7	-3.1	-
AA, 2 ip, qd 6-14			33	-	-	-	0/10	0.2	1.6	-2.1	-
Vincr ine, 0.22, ip, qd 6-14 AA, 2 ip, qd 6-14			38	-	-	-	0/10	-	1.9	-1.9	Scant Data
Contr : MDD = 10.5; there were no tumor-free survivors among the 20 control mice. Td = 0.64 days. Expt. 1632 PS-CC-119.											
Vincr stine, 1.9, iv, 3,7,11,15,19	Mammary 16/C (IV)	Early (10^6 cells)	56	-	-	-	0/10	0.3	1.6	0.2	-
DiOHA, 1.5, sc, qd 3-19			72	-	-	-	0/10	0.1	1.2	-0.2	-
Vincristine, 0.73, iv, 3,7,11,15,19 DiOHA, 0.93, sc, qd 3-19			92	-	-	-	0/10	-	2.0	0.6	~1.6
Control: MDD = 25.0; there were no tumor-free survivors among the 20 control mice. Td = 3.5 days. Expt. 1623 F1.											
DTIC, 135, po, 19,21,23,25,27,29	Colon 11/A (SC)	Advanced (100-400 mg)	121	46.4	9/10	4/10	0/10	0.6	3.7	2.9	-
DiOHA, 1.4, iv, 19,21,23,25,27,29			23	20.5	1/10	1/10	1/10	0.3	1.6	0.8	-
DTIC, 90, po, 19,21,23,25,27,29 DiOHA, 1.4, iv, 19,21,23,25,27,29			207	84.6	10/10	8/10	0/10	-	6.7	5.9	-
DTIC, 90, po, 19,21,23,25,27,29 DiOHA, 1.0, iv, 19,21,23,25,27,29			203	73.3	9/10	6/10	0/10	-	5.8	5.0	1.5
Control: MDD = 45.5; time for median tumor to reach 1250 mg = 27.5 days; there were no tumor-free survivors among the 20 control mice. Td = 3.8 days. Expt. 1708 C1.											
DTIC, 135, po, 18,20,22,24	Colon 11/A (SC)	Advanced (100-400 mg)	28	41.4	8/10	0/10	0/10	0.8	3.1	2.7	-
DiOHA, 1.4, iv, 18,20,22,24			-7	18.6	3/10	0/10	0/10	0.4	1.4	1.0	-
DTIC, 90, po, 18,20,22,24 DiOHA, 1.0, iv, 18,20,22,24			43	46.4	11/11	4/11	0/11	-	3.5	3.0	1.3
Control: MDD = 86.5; time for median tumor to reach 1250 mg = 33.4 days; there were no tumor-free survivors among the 20 control mice. Td = 4.0 days. Expt. 1708 C2.											
L-PAM, 10.0, ip, 30,36,42	Mammary 14 (SC)	Advanced (100-500 mg)	63	26.5	3/10	2/10	0/10	1.2	3.6	2.0	-
DiOHA, 4.8, sc, 30,36,42			15	12.0	1/10	0/10	0/10	0.6	1.7	0.0	-
L-PAM, 6.2, ip, 30,36,42 DiOHA, 3.8, sc, 30,36,42			39	36.9	8/10	2/10	0/10	-	5.0	3.4	1.15
Cont l: MDD = 76.0; time for median tumor to reach 1250 mg = 41.0 days; there were no tumor-free survivors among the 20 control mice. Td = 2.2 days. Expt. 1712 Q1.											
cis- t, 6.0, ip, 6,10,14	P388 (IP)	Advanced (10^6 cells)	77	-	-	-	0/10	1.8	5.3	0.3	-
DiOH 6.0, ip, 6,10,14			136	-	-	-	0/10	3.1	9.4	4.4	-
cis- t, 6.0, ip, 6,10,14 DiOH 2.7, ip, 6,10,14			190	-	-	-	3/10	-	13.2	8.2	~1.4
Cont : MDD = 11.0; there were no tumor-free survivors among the 20 control mice. Td = 0.48 days. Expt. 1657 PS-CC-146.											

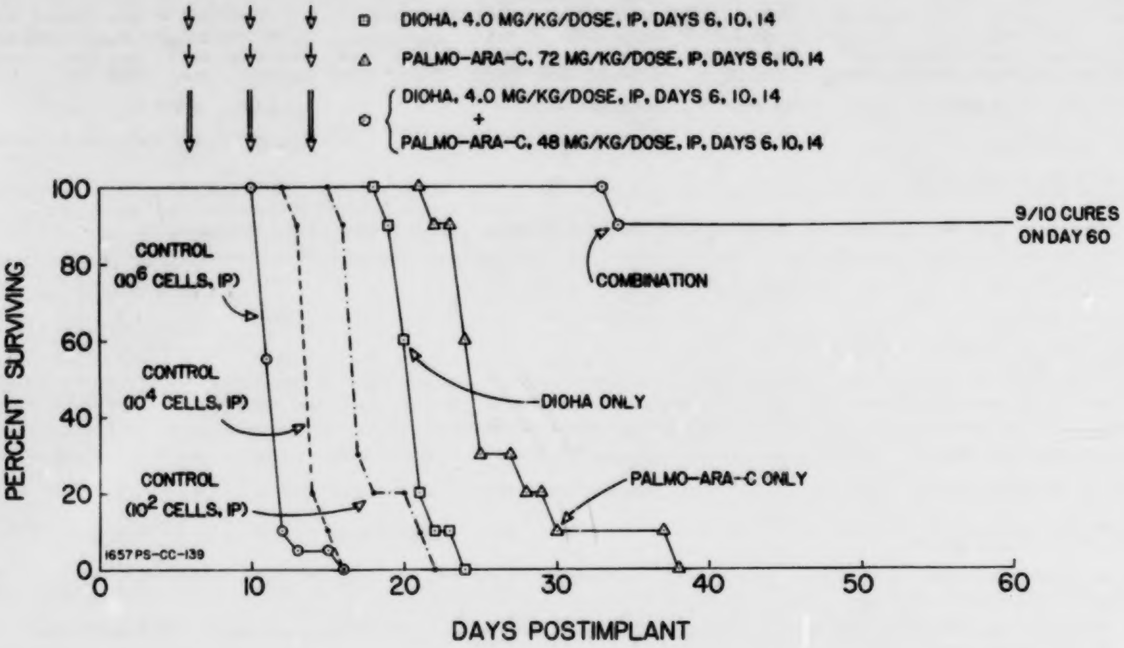

FIGURE 2.—DiOHA and palmO-ara-C treatment of advanced-stage, intraperitoneally implanted P388 leukemia in male CDF$_1$ mice. Doses shown are highest nontoxic doses and all treatments were carried out against 10^6 cells.

TABLE 3.—Determination of CTI for the combination of DiOHA + palmO-ara-C (Experiment 1672P1)

			Actual deaths‡ observed in—	
Agent	Doses* used in the combinations	Fraction of LD10 dose†	Tumor mice (n = 10)	Toxicity control mice (n = 10)
DiOHA	2.6	1.73		
PalmO-ara-C	21	0.91		
		2.64	10 (LD100)	9 (LD90)
DiOHA	1.7	1.13		
PalmO-ara-C	13	0.56		
		1.69	6 (LD60)	3 (LD30)
DiOHA	1.7	1.13		
PalmO-ara-C	8	0.35		
		1.48	4 (LD40)	4 (LD40)
DiOHA	1.7	1.13		
PalmO-ara-C	5.2	0.23		
		1.36	4 (LD40)	1 (LD10)
DiOHA	1.1	0.73		
PalmO-ara-C	13	0.56		
		1.29	1 (LD10)	0 (LD0)
DiOHA	1.1	0.73		
PalmO-ara-C	8	0.35		
		1.08	0 (LD0)	1 (LD10)

*DiOHA, q2d × 5, iv; palmO-ara-C, ip, daily × 10.

†As determined from fig 3: DiOHA = 1.5 mg/kg/dose; palmO-ara-C = 23 mg/kg/dose.

‡Deaths within 30 days after last treatment.

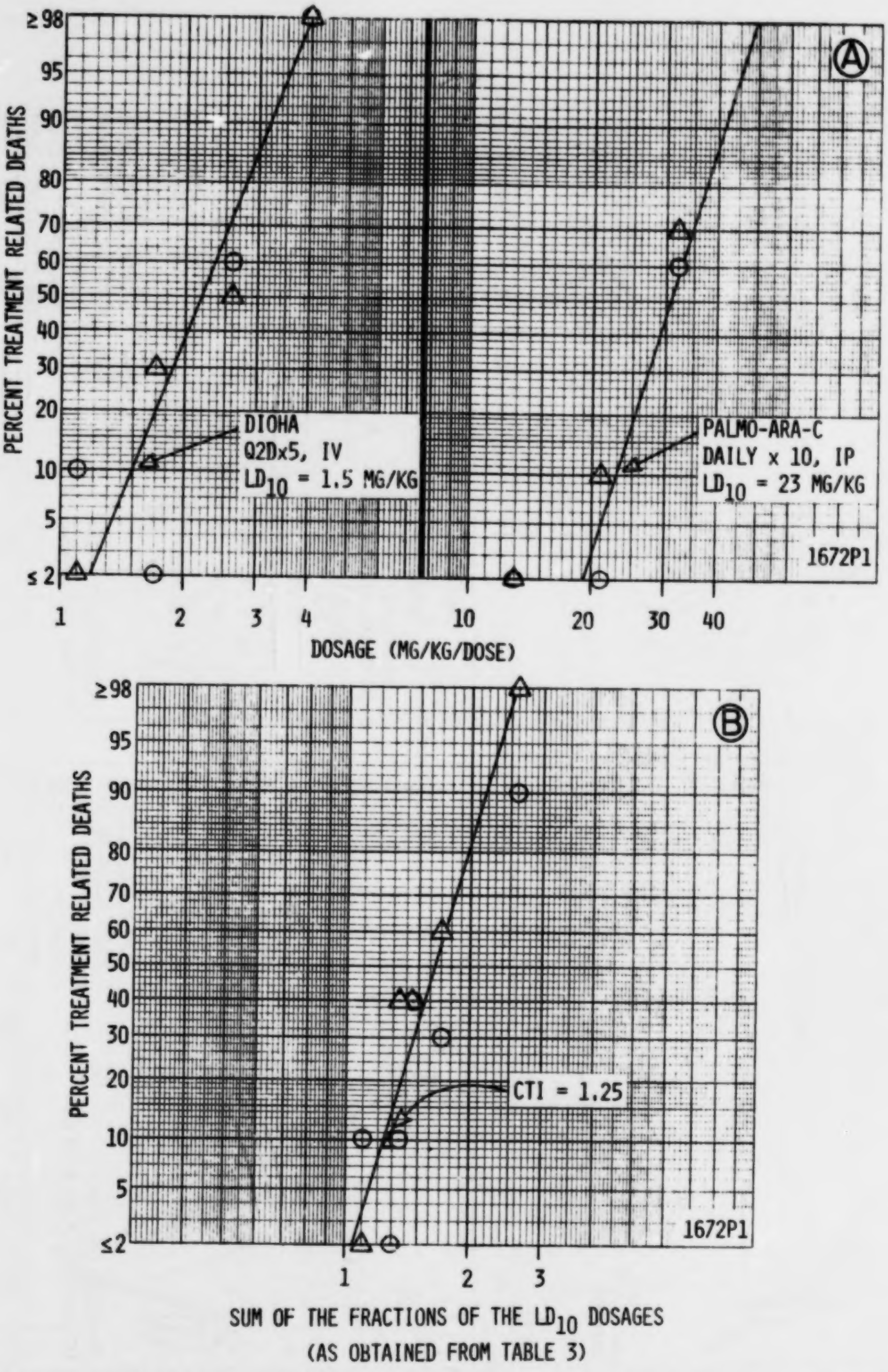

FIGURE 3.—A. Determination of LD10 values for DiOHA and palmO-ara-C in male CDF_1 mice. B. Determination of CTI for DiOHA and palmO-ara-C in male CDF_1 mice. △ = drug deaths of tumor mice (10/group); ⊙ = drug deaths of nontumor, toxicity control mice (10/group) (*see* fig 3A and table 3).

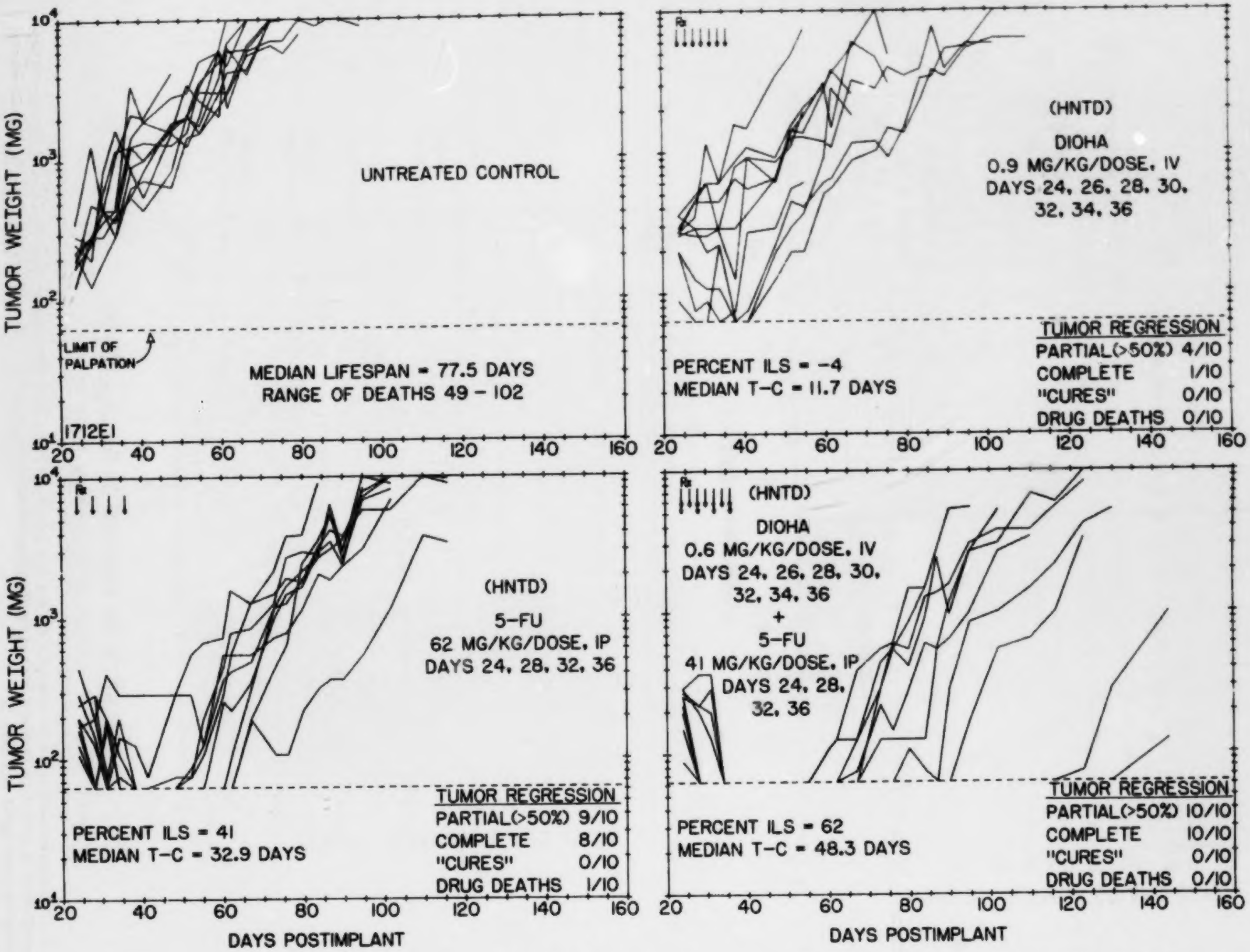

FIGURE 4.—DiOHA and 5-FU treatment of subcutaneously implanted mammary adenocarcinoma 14 in male B6C3F$_1$ mice. Tumors were 100–400 mg at the time of 1st treatment. HNTD = highest nontoxic dose.

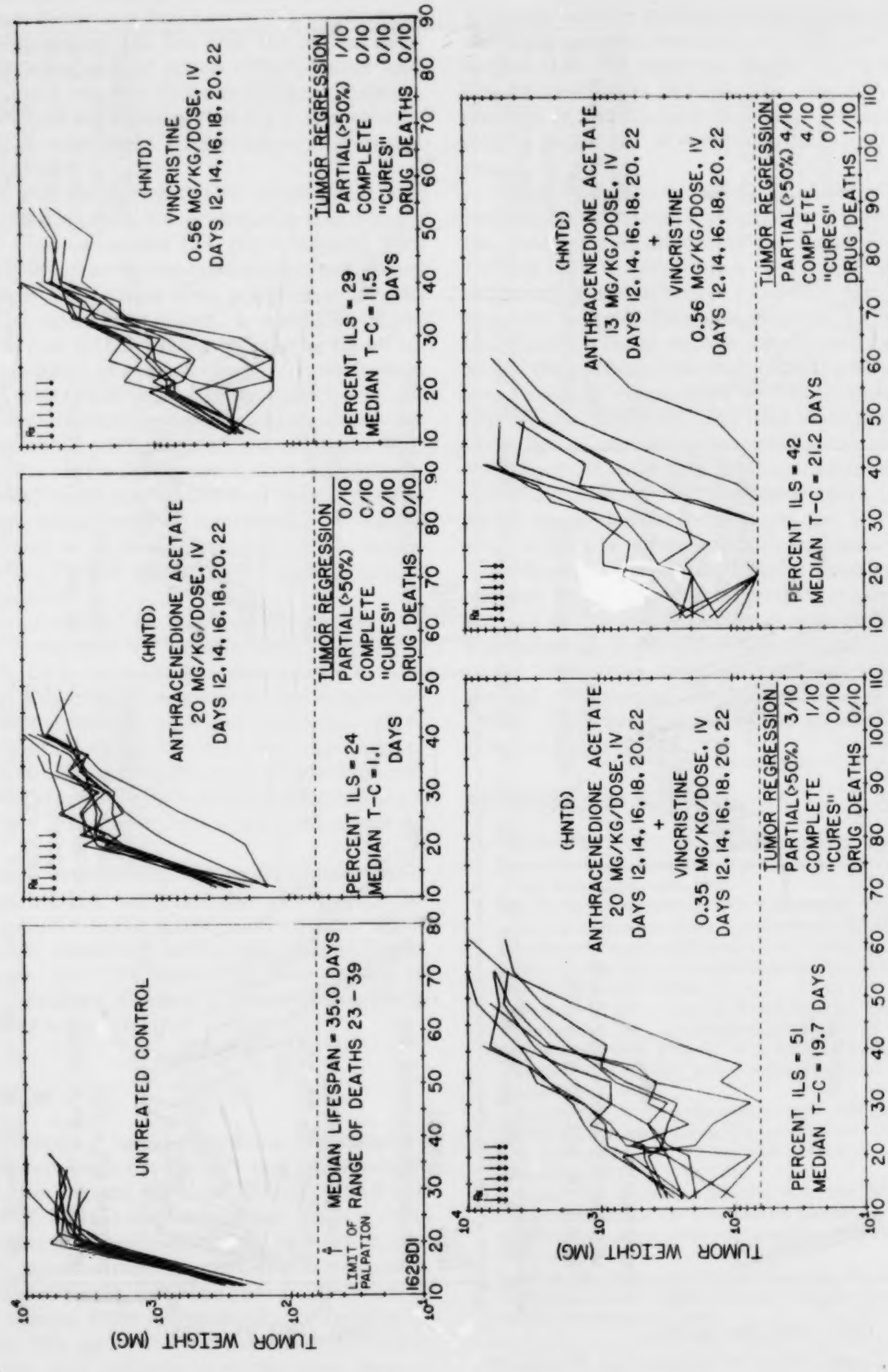

FIGURE 5.—AA and VCR treatment of subcutaneously implanted mammary adenocarcinoma 13/C in male B6C3F$_1$ mice. Tumors were 100–400 mg at the time of 1st treatment. HNTD = highest nontoxic dose.

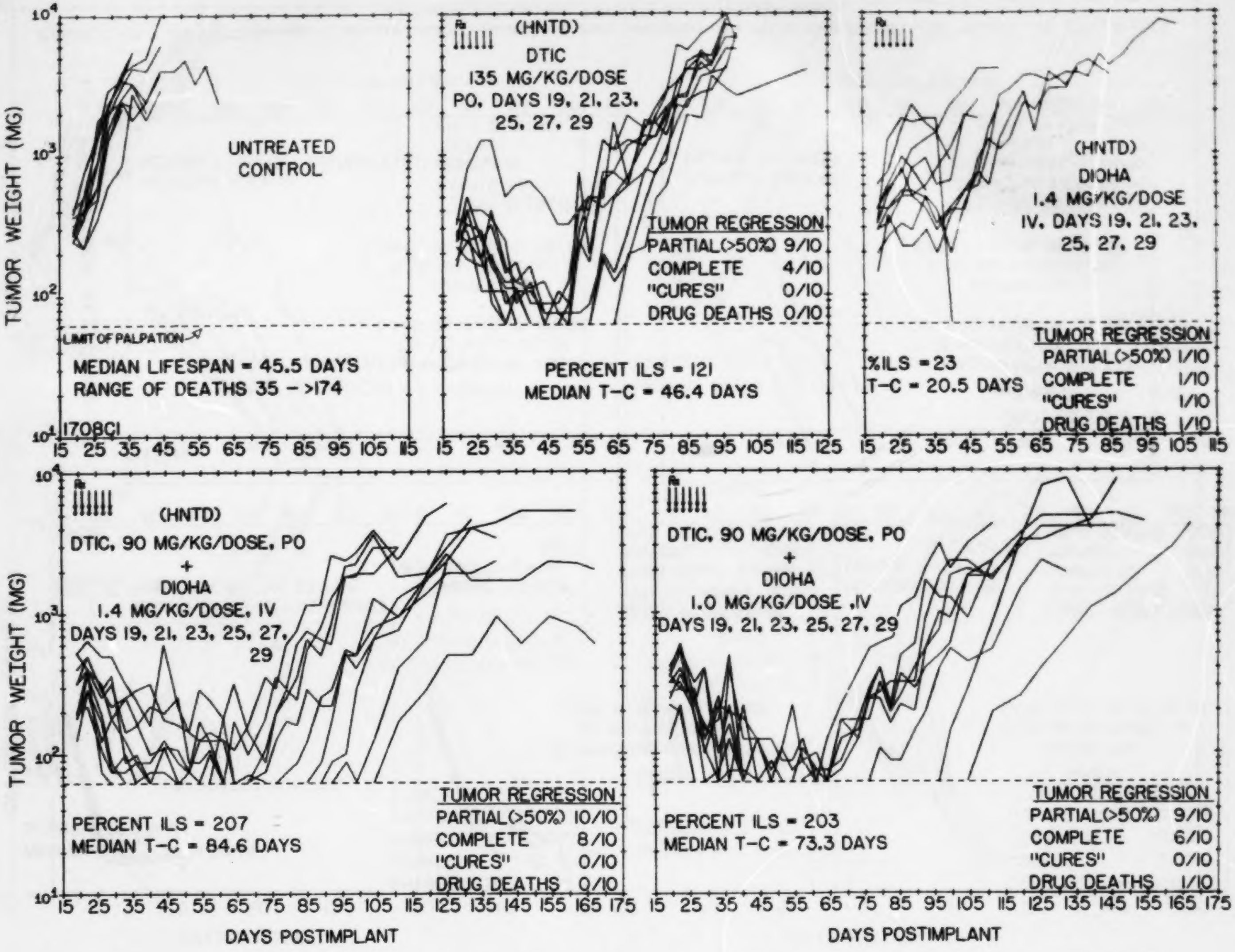

FIGURE 6.—DiOHA and dacarbazine treatment of subcutaneously implanted colon adenocarcinoma 11/A in male CDF_1 mice. Tumors were 100–400 mg at the time of 1st treatment. HNTD = highest nontoxic dose.

dose of each agent can be used in combination. Although cross-resistance data have not been obtained for this combination, the fact that the agents have differing mechanisms of action (DNA binder and antimetabolite) renders cross-resistance unlikely. Previous results have shown that 5-FU is not cross-resistant with other DNA binders (dactinomycin and doxorubicin) (12).

The AA and vincristine (VCR) combination was found to have modest TS against advanced-stage mammary adenocarcinoma 13/C (fig 5, table 2). Two different dose ratios for the combination are shown in this figure; both ratios were superior to the optimum single-agent treatments, as evidenced by improved T-C and %ILS values, as well as by an increased frequency of regressions. The combination of DiOHA and VCR was slightly superior to the optimum use of either agent alone against intravenously implanted mammary adenocarcinoma 16/C (table 2). The CTI values were 1.6 for both experiments, which indicates that 80% of the full dose of each agent can be used in combination. A line of P388 resistant to VCR was as sensitive to DiOHA as the parent P388/O tumor, indicating the lack of cross-resistance.[6]

The combination of DiOHA and dacarbazine had moderate to marked TS against colon adenocarcinoma 11/A (fig 6, table 2). An improvement in T-C, %ILS, and frequency of regression was evident for the combination groups in both experiments (fig 6, table 2). The CTI values for these two experiments were 1.3 and 1.5 (table 2). Because of their different mechanisms of action (alkylation and DNA binding), it is unlikely that these two agents are cross-resistant.

In a single experiment, the combination of melphalan and DiOHA was evaluated and found to be modestly superior to the optimum use of either agent alone against mammary adenocarcinoma 14 (table 2). However, the CTI value was a very low 1.15 (table 2), indicative of nearly a complete overlap in dose-limiting toxic effects.

DISCUSSION

Four promising anthracenedione combinations have been reported: (*a*) DiOHA and palmO-ara-C; (*b*) DiOHA or AA and 5-FU; (*c*) DiOHA or AA and VCR; and (*d*) DiOHA and dacarbazine. Each of these combinations was demonstrated to have TS in confirmatory experiments. An additional combination, DiOHA and cisplatin, has been previously shown to have TS against P388 leukemia (4). Interestingly, these same five agents: palmO-ara-C, 5-FU, VCR,[6] dacarbazine, and cisplatin have also been demonstrated to have TS with doxorubicin (4,9,13–15). Although testing was restricted to colon and mammary adenocarcinomas and to L1210 and P388 leukemias, the TS observed should be applicable to other tumor types in man. For example, the combination of DiOHA and dacarbazine may be useful for the treatment of soft tissue sarcomas or melanomas.

Due to a similar mechanism of action, it is perhaps not unexpected that P388 leukemia cells selected for resistance to doxorubicin are also resistant to DiOHA (12). In spite of this fact, many tumors that are innately unresponsive to or only marginally responsive to doxorubicin are sensitive to the anthracenediones.[7] These include colon adenocarcinomas 38 and 06/A. This would imply that if a human tumor was innately responsive to doxorubicin (tumor regressions observed), and then eventually regrew in the face of continuing doxorubicin treatment (drug resistance), it may be a poor candidate for anthracenedione therapy. On the other hand, if a tumor never responded to doxorubicin (innately insensitive), it may be an acceptable candidate.

It should be emphasized that a combination usually has TS only against a tumor that is responsive to each of the cytotoxic drugs used singly (8–10). Furthermore, if one of the agents is clearly superior to the other, then the dose ratio also becomes important. In general, the highest ratio of the most active of the two agents should be used to obtain the maximum TS (16,17).

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Combinations of 5-FU, Hypoxanthine, and Allopurinol in Chemotherapy for Human Colon Adenocarcinoma Xenografts[1,2]

Janet A. Houghton* and Peter J. Houghton[3,4]

SUMMARY

A series of four human colon adenocarcinomas, growing as xenografts in immune-deprived mice, have been used to evaluate the efficacy of 5-FU in combination with two purines, hypoxanthine (Hx) and allopurinol (HPP), which have reduced the toxicity of 5-FU in host mice. Tumor-bearing mice were treated at 7-day intervals with 5-FU administered simultaneously with the protecting agents (Hx and HPP). Two tumor lines ($HxVRC_5$ and $HxGC_3$), insensitive to 5-FU alone, failed to show any response to this combination. In 5-FU-sensitive $HxELC_2$ tumors, the combination of 5-FU with Hx and HPP did not increase the therapeutic index, and in $HxHC_1$ xenografts, antagonism to 5-FU cytotoxicity was observed. Tumor response in relation to the pathways of 5-FU metabolism is discussed.

[Cancer Treat Rep 66:1201–1206, 1982]

Allopurinol (HPP) has protected normal tissues from the toxicity of 5-FU experimentally in rodents (1,2) and in man (3,4), either by prior or simultaneous administration. Such protection has been reported for both gastrointestinal (GI) tissues and bone marrow (1,2). Hypoxanthine (Hx) has also reduced 5-FU toxicity in these tissues, particularly in the bone marrow, when given by simultaneous administration either alone or in combination with HPP (1).

HPP (5) and Hx (6,7) have antagonized the cytotoxicity of 5-FU in cultured cell lines. This effect of HPP and its protection of normal host tissues has been attributed to the accumulation of orotic acid caused by the inhibition of orotidine 5′-monophosphate decarboxylase by metabolites of HPP (5). Orotic acid may then compete favorably with 5-FU for orotate phosphoribosyltransferase [OPRTase; (8)], a key enzyme in the metabolism of 5-FU to ribonucleotides. The effects produced by Hx have, however, been attributed to the depletion of 5-phosphoribosyl-1-pyrophosphate [PRPP (6,7,9)], the ribose phosphate donor required for the conversion of 5-FU to FUMP by OPRTase. Other cell lines in culture (5) together with some human colorectal tumors (9) appear to metabolize 5-FU to FUMP, predominantly by the sequential activities of uridine phosphorylase (UP) and uridine kinase (UK), utilizing ribose-1-phosphate (R-1-P), and thus should not be affected by treatment with either Hx or HPP. Hence, if normal tissues can be protected by these agents, then a basis for selectivity exists for combining such agents with 5-FU in an attempt to increase the therapeutic index for the treatment of those tumors that may metabolize 5-FU predominantly by UP and UK.

Treatment with combinations of HPP and 5-FU have been reported in clinical trials (3,4,10). Pretreatment and subsequent administration of HPP have allowed a twofold higher dose of 5-FU to be given (3,4), resulting in a fourfold increase in the concentration times time exposure to 5-FU by continuous infusion (10). Favorable responses in six of 12 patients with GI cancer have been reported (3),

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[1] Received July 13, 1981; revised Dec 9, 1981; accepted Dec 17, 1981.

[2] Supported by Public Health Service grant CA-21677 (National Large Bowel Cancer Project) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, by American Cancer Society grant CH-172, and by ALSAC.

[3] Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, TN.

[4] We acknowledge the technical assistance of Ms. Kim Cassin and Ms. Joan Brown.

**Reprint requests to*: Janet A. Houghton, PhD, Division of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, 332 N Lauderdale, PO Box 318, Memphis, TN 38101.

while other investigators have detected no activity in patients with colon carcinoma (4,10).

Because both Hx and HPP were shown to protect normal tissues, and since HPP inhibits the degradation of Hx, this combination was considered to be of interest for administration with 5-FU in the chemotherapy for colon carcinoma.

The antitumor effectiveness of 5-FU in combination with Hx and HPP has therefore been examined in four of these tumor lines. Two tumors previously demonstrated high R-1-P/PRPP ratios (~ 5) and appeared to metabolize 5-FU predominantly by the UP-UK pathway, while the other two lines possessed a lower ratio (~ 1) and utilized OPRTase and PRPP preferentially in the biosynthesis of FUMP from 5-FU (9). One line in each of the two groups has previously demonstrated some sensitivity to treatment with 5-fluoropyrimidines (11). This study reports on the biologic testing of 5-FU in combination with Hx and HPP by simultaneous administration.

MATERIALS AND METHODS

Chemicals

5-FU was obtained from Hoffmann-LaRoche, Inc, Nutley, NJ, and Hx and HPP were purchased from Sigma Chemical Co, St. Louis, MO.

Immune Deprivation of Mice

Four-week-old female CBA/CaJ mice were immune-deprived by thymectomy, lethal whole-body irradiation, and reconstitution with syngeneic bone marrow as previously described (11).

Toxicity Studies

Hx and HPP were dissolved in the smallest possible volume of 1 M KOH and, after dilution, were back titrated with 1 M HCl as previously described (1). The toxicity of a single dose of 400 mg/kg ip of 5-FU with or without the simultaneous ip administration of various doses of Hx (50, 100, or 200 mg/kg), of HPP (10, 20, or 40 mg/kg), or both, was determined as previously reported (1,12). Normal male CBA/CaJ mice (Jackson Laboratory, Bar Harbor, ME), 8–12 weeks of age, were injected on Day 0, and the number of drug-related deaths was assessed over a period of 20 days. The animals were caged in an air-conditioned room, which was lighted for 12 hours each day. Cages, litter, and food (Purina 5010) were autoclaved and mice were placed in fresh cages twice weekly. Weight loss obtained in groups of mice in which no deaths were observed has been reported previously (1).

Measurement of Response to Chemotherapy

Two tumor pieces, approximately 5 mm^3, were implanted sc into the dorsal flanks of each immune-deprived mouse. This procedure resulted in the growth of two discrete tumors. Mice were randomized into groups of up to 13 and were treated when tumors were established and had reached approximately 9–12 mm in diameter. Tumor-bearing mice received either 25 or 50 mg/kg of 5-FU alone, or 50, 75, or 100 mg/kg of 5-FU simultaneously with 50 mg/kg of Hx and 10 mg/kg of HPP, by ip injection, at 7-day intervals. Tumor growth was assessed from the measurement of two perpendicular diameters, at weekly intervals, using vernier calipers. Since tumor shape approximated that of a sphere, tumor volume was calculated by substitution into the formula $\pi/6 \times d^3$, where d is the mean diameter. The delay in growth of treated tumors was assessed when these tumors had reached four times their treatment volume, as previously described (13). The four lines of human colorectal adenocarcinoma used in this study have been extensively characterized and their responsiveness to 5-fluoropyrimidines has been reported (11,13–16).

RESULTS

The toxicity of 5-FU at a dose level of 400 mg/kg administered either alone or in combination with various doses of Hx and HPP is shown in table 1. 5-FU alone was lethal in four of five mice; however, Hx at doses of 50 and 100 mg/kg, HPP at all dose levels examined, and the low-dose and intermediate-dose levels of the purine combination, when administered simultaneously with 400 mg/kg of 5-FU, reduced the 5-FU-induced deaths observed. No deaths were observed in those groups receiving either 50 mg/kg of Hx, 10 mg/kg of HPP, or these two low doses of each purine in combination with 5-FU. In each of the four remaining groups in which some protection was observed, two of five mice died. Mice receiving the low-dose purine combination have demonstrated less weight loss than those treated with either 50 mg/kg of Hx or 10 mg/kg of HPP administered simultaneously with 5-FU (1). This dose level of the purine combination was therefore selected for subsequent use in growth inhibition studies.

In tumor lines $HxGC_3$ and $HxVRC_5$, 5-FU administered at a dose level of 50 mg/kg/week did not cause a delay in tumor growth (fig 1). Similarly,

TABLE 1.—Toxicity studies in non-tumor-bearing mice*

Dose (mg/kg)			
5-FU	Hx	HPP	No. dead/total
400	—	—	4/5
400	50	—	0/4
		—	
400	100	—	2/5
400	200	—	5/5
400	—	10	0/4
400	—	20	2/5
400	—	40	2/5
400	50	10	0/5
400	100	20	2/5
400	200	40	4/5

*All agents were administered simultaneously ip.

when either 50 or 75 mg/kg of 5-FU was injected simultaneously with Hx and HPP at weekly intervals, no growth inhibition was obtained.

In $HxELC_2$ tumors (fig 1, table 2), 5-FU, at a dose level of 25 mg/kg, failed to induce growth inhibition after weekly administration to tumor-bearing mice, while 50 mg/kg of 5-FU, administered on the same schedule, induced a delay in tumor growth of 17 days ($\log_{10}$ tumor cell kill of 0.33). In combination with Hx and HPP, the growth inhibition induced by 5-FU at the dose level of 50 mg/kg was identical to that obtained with the same dose of 5-FU administered alone. As the dose of 5-FU was increased to 75 or 100 mg/kg in the presence of Hx and HPP, a delay in tumor growth of 24 and 21 days, respectively, was obtained. These values, together with the $\log_{10}$ tumor cell kill (0.48 and 0.42, respectively), were not significantly different from those obtained with 5-FU at the dose level of 50 mg/kg.

The growth inhibition obtained in $HxHC_1$ tumors was also examined (fig 1, table 3). Similar to observations made in $HxELC_2$ tumors, 5-FU, at a dose level of 25 mg/kg, produced very little growth inhibition (9 days). At a dose of 50 mg/kg, administered at weekly intervals, 5-FU inhibited the growth of tumors for 28 days ($\log_{10}$ tumor cell kill of 0.60), while the same dose level of 5-FU, when given in combination with Hx and HPP, reduced the growth inhibition achieved to the level obtained following the administration of 25 mg/kg of 5-FU alone. Increasing the dose of 5-FU to either 75 or 100 mg/kg in the presence of Hx and HPP produced a delay in tumor growth similar to that obtained with 50 mg/kg of 5-FU administered alone (27 and 33 days, respectively, or 0.57 and 0.69 estimated as the $\log_{10}$ tumor cell kill); growth inhibition was not increased significantly at these two dose levels.

Tumor-bearing mice treated sequentially with 5-FU at a dose level of 100 mg/kg did not survive.

DISCUSSION

We have demonstrated that Hx, HPP, or a combination of these two purines, at low doses, can protect mice from the toxic effects caused by potentially lethal doses of 5-FU when administered simultaneously. These data are similar to those reported by others for 5-FU in the presence of HPP alone, both in rodents (2) and in man (3,4). The mechanism of 5-FU toxicity to the GI tract in mice has been correlated with its metabolism and incorporation into RNA (12). It was reported (17) that incorporation of 5-FU into the RNA of bone marrow and GI tissue of CF_1 mice was reduced by 45% and 67%, respectively, in the presence of HPP. It is possible that the depletion of PRPP may be responsible for these findings (1,9); such a depletion has been identified in HPP-treated human erythrocytes both in vivo and in vitro (18).

Tumor lines $HxGC_3$ and $HxVRC_5$ have been insensitive to treatment with a single, maximum tolerated dose of 5-FU (11); weekly injection of 5-FU alone or at a higher dose in combination with Hx and HPP did not induce response in either of these two tumors. In 5-FU-sensitive $HxELC_2$ tumors, administration of Hx and HPP with both 75 and 100 mg/kg of 5-FU did not significantly increase the delay in tumor growth achieved with 50 mg/kg of 5-FU, administered alone. In $HxHC_1$ tumors, however, 50 mg/kg of 5-FU administered with Hx and HPP was less effective than was 50 mg/kg of 5-FU alone. Increasing the dose of 5-FU to 75 and 100 mg/kg in the presence of Hx and HPP did not increase the growth inhibition over that achieved with

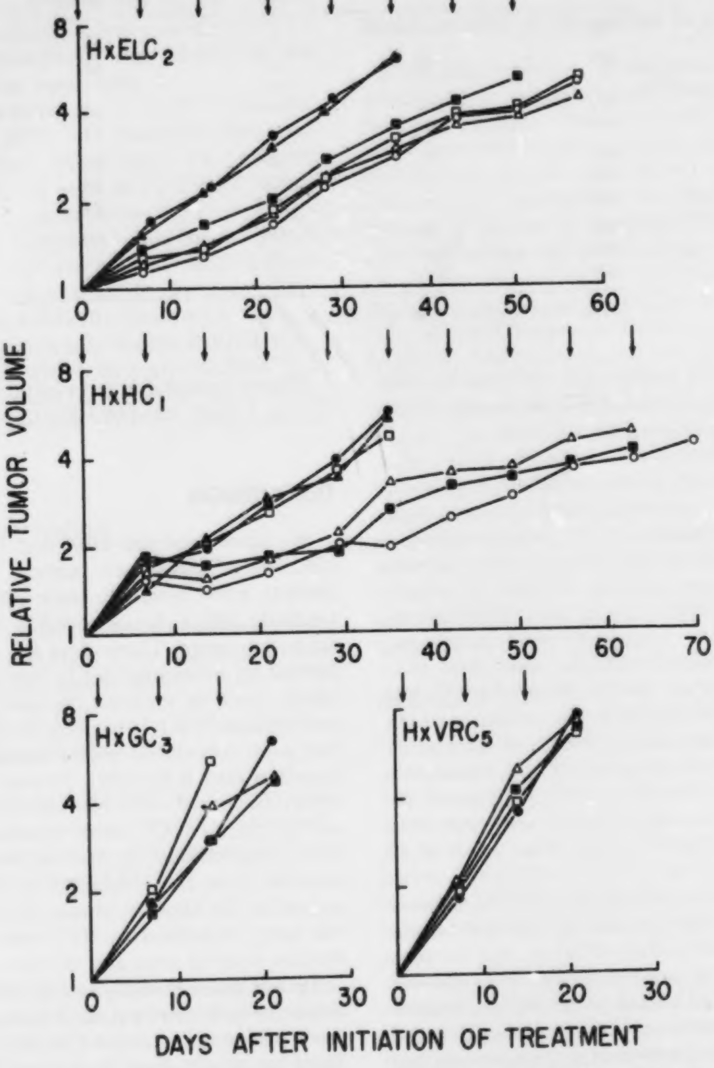

FIGURE 1.—5-FU was administered ip to tumor-bearing mice at various doses either alone or in combination with Hx (50 mg/kg) and HPP (10 mg/kg). Drugs were injected simultaneously at 7-day intervals, and growth inhibition of tumors was determined as described in the Materials and Methods section. ● = control; ▲ = 25 mg/kg of 5-FU and ■ = 50 mg/kg of 5-FU; □ = 50 mg/kg of 5-FU, △ = 75 mg/kg of 5-FU, and ○ = 100 mg/kg of 5-FU, all administered with Hx and HPP.

TABLE 2.—Responsiveness of tumor line $HxELC_2$

Dose (mg/kg)		Tumor diameter (mm) at 1st treatment†	Time (days) to 4 × treatment volume†	Growth delay (days)	Log_{10}‡ tumor cell kill	No. of drug-related deaths	Total No. of mice
5-FU	Hx and HPP*						
0	—	11 ± 2	30.6 ± 10.3	—	—	0	12
25	—	11 ± 2	32.9 ± 16.8	2.3	0.06	1	11
50	—	11 ± 2	47.6 ± 27.6	17.0	0.33	1	12
50	50 and 10	10 ± 1	48.6 ± 17.5	17.6	0.36	3	12
75	50 and 10	10 ± 2	54.5 ± 19.0	23.9	0.48	1	13
100	50 and 10	11 ± 3	51.9 ± 13.5	21.3	0.42	6	13

*Hx and HPP were administered simultaneously with 5-FU at weekly intervals.

†Values = mean ± 1 SD for each group.

‡Log_{10} tumor cell kill was calculated by the method of Griswold et al (19). Volume doubling time of untreated tumors was 15.3 ± 5.1 days.

TABLE 3.—Chemotherapy for $HxHC_1$ tumors

Dose (mg/kg)		Tumor diameter (mm) at 1st treatment†	Time (days) to 4 × treatment volume†	Growth delay (days)	Log_{10}‡ tumor cell kill	No. of drug-related deaths	Total No. of mice
5-FU	Hx and HPP*						
0	—	10 ± 3	28.5 ± 9.2	—	—	0	6
25	—	11 ± 3	37.4 ± 11.7	8.9	0.18	0	5
50	—	12 ± 2	56.6 ± 34.6	28.1	0.60	1	6
50	50 and 10	10 ± 4	37.7 ± 17.3	9.2	0.18	1	6
75	50 and 10	10 ± 2	55.0 ± 28.5	26.5	0.57	1	6
100	50 and 10	11 ± 3	61.7 ± 24.4	33.2	0.69	1	6

†Values = mean ± 1 SD for each group.

‡Log_{10} tumor cell kill was calculated by the method of Griswold et al (19). Volume doubling time of untreated tumors was 14.3 ± 4.6 days.

the maximum tolerated dose of 5-FU administered alone.

In a prior study, it was demonstrated that in lines HxELC$_2$ and HxHC$_1$, depletion of PRPP to between 21% and 29% of control levels occurred during the first 15 minutes after administration of Hx (50 mg/kg) and HPP (10 mg/kg) in combination to tumor-bearing mice (9). In addition, no orotic acid was detected for up to 4 hours after treatment. This effect correlated with the decreased concentration of fluorinated ribonucleotides observed in HxHC$_1$ tumors after purine treatment, while the concentrations of these nucleotides in HxELC$_2$ tumors remained unchanged in the presence of Hx and HPP; 5-FU, however, was retained in higher concentrations in each tumor line after administration of the three-agent combination (1,9). HxHC$_1$ and HxVRC$_5$ tumors demonstrated relatively low levels of 5-FU, high concentrations of 5-FU ribonucleotides, and a low R-1-P/PRPP ratio; HxELC$_2$ and HxGC$_3$ tumors possessed higher levels of 5-FU, lower levels of these nucleotides, and a high R-1-P/PRPP ratio (9). Lines HxHC$_1$ and HxVRC$_5$ appeared, therefore, to metabolize 5-FU preferentially by OPRTase, while HxELC$_2$ and HxGC$_3$ tumors used predominantly the alternative pathway for ribonucleotide biosynthesis that utilized R-1-P. Antagonism to 5-FU cytotoxicity was observed in HxHC$_1$ tumors after administration of Hx and HPP. Increasing the dose of 5-FU in the presence of the two purines restored the antitumor effect but did not increase it. After treatment of mice bearing HxELC$_2$ tumors with Hx, HPP, and 5-FU, no antagonism was observed, but neither was an increased antitumor effect evident, even when 100 mg/kg of 5-FU was administered with the two purines. It is apparent that patients who presented with colon carcinomas that utilized the OPRTase pathway (eg, HxHC$_1$) predominantly in 5-FU metabolism may be compromised if treated with the 5-FU-HPP combination. Our studies have demonstrated that 5-fluoropyrimidine-sensitive tumors can metabolize 5-FU by utilizing either PRPP or R-1-P. Hence, it is possible that administration of the combination may reduce the overall response rate of 20% observed with the use of 5-FU administered alone. Using the xenograft model, we have been unable to demonstrate an increased therapeutic effect by combining 5-FU with Hx and HPP.

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Brief Reports

Combined Chemotherapy With Doxorubicin, Bleomycin, Vinblastine, Dacarbazine, and Radiotherapy for Advanced Lymphoepithelioma[1]

Enzo Galligioni,[2,*] Antonino Carbone,[3] Umberto Tirelli,[2] Andrea Veronesi,[2] Mauro G. Trovò,[2] M. Donatella Magri,[2] Diana Crivellari,[2] Mario Roncadin,[2] Sergio Frustaci,[2] Salvatore Tumolo,[2] and Eligio Grigoletto[2]

SUMMARY

Twelve consecutive patients with advanced lymphoepithelioma and no previous chemotherapy or radiotherapy were observed in our Division between January 1975 and December 1980. Primary treatment of 11 evaluable patients with doxorubicin (Adriamycin), bleomycin, vinblastine, and dacarbazine (ABVD) for at least three cycles resulted in complete remission (CR) in four patients; partial remission with a > 50% reduction of tumor volume in four; partial remission with a < 50% reduction of tumor volume in one; and no response in two. With the addition of radiotherapy in doses of 4500–6000 rad to the primary tumor and the bilateral laterocervical lymph node chains, the combined treatment resulted in CR in eight patients. At 27 months the median duration of CR in these patients has not been achieved, and the median survival has not been achieved at 32 months.

[Cancer Treat Rep 66:1207–1210, 1982]

Radiotherapy (RT) fails to cure most patients with advanced lymphoepithelioma (1–3). Chemotherapy (CT) has been mostly used with palliative function in recurrences and metastases; among single agents, doxorubicin (Adriamycin) and cyclophosphamide have been reported to yield a 40% response rate, whereas bleomycin, methotrexate, 5-FU, and vincristine showed a slightly inferior efficacy (3,4). In advanced lymphoepithelioma, combination CT as first therapy was employed by investigators at the M. D. Anderson Hospital and Tumor Institute, Houston, TX, in four evaluable patients and resulted in complete remission (CR) in three patients and partial remission (PR) in one; durations of remission were not reported (4). Doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) is an effective and tolerable regimen in Hodgkin's disease (5). It consists of at least three drugs singly effective in lymphoepithelioma, and synergistic activity between doxorubicin and dacarbazine has been suggested (6,7). To evaluate the efficacy and toxicity of combination CT as primary treatment and the feasibility of a chemoradiotherapeutic regimen in advanced lymphoepithelioma, we undertook a clinical study using ABVD followed by RT; the preliminary results have been presented previously (8).

PATIENTS AND METHODS

Between January 1975 and December 1980, 12 consecutive patients (seven males and five females) with a median age of 46 years (range, 16–57) entered this study. All patients had stage IV disease according to the TNM classification (9), with involvement of the bilateral or fixed laterocervical lymph nodes (seven patients) or base of the skull (one) or with distant metastases (four). Entry in the study was restricted to those patients with no previous CT or RT and with adequate renal, hepatic, pulmonary, cardiac, and hematopoietic functions. Histopathologic diagnosis was established according to the recommended criteria, (10–12) on the basis of nasopharyngeal or tonsillar tissue biopsies in seven and two patients, respectively. In the remaining three patients, the diagnosis of metastases from lymphoepithelioma was ascertained by surgical lymph node biopsy, and primary tumor was later found in

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[1] Received May 7, 1981; revised Nov 12, 1981; accepted Dec 2, 1981.

[2] Division of Radiotherapy and Medical Oncology, Ospedale Civile, Pordenone, Italy.

[3] Department of Pathology, Istituto Scientifico per lo Studio e la Cura dei Tumori, Istituto di Oncologia della Università, Genova, Italy.

Reprint requests to: Dr. Enzo Galligioni, Division of Radiotherapy and Medical Oncology, Ospedale Civile, 33170 Pordenone, Italy.

Table 1.—Patient characteristics, response, and survival*

Patient No.	Sex	Age (yrs)	Primary site	Stage	EBV	Response to ABVD	Response to ABVD + RT	Duration of response (mos)	Survival from diagnosis (mos)
1	M	56	Tonsil	IV ($T_2N_2M_0$)	–	CR	CR	43+	46+
2	F	47	Nasopharynx	IV ($T_2N_3M_0$)	–	PR†	CR	10	28+
3	M	35	Tonsil	IV ($T_2N_3M_0$)	–	CR	CR	31+	36+
4	F	49	Nasopharynx	IV ($T_2N_3M_0$)	+	NR	CR	34	54
5	F	27	Nasopharynx	IV ($T_2N_2M_0$)	+	PR‡	CR	38	60+
6	F	52	Nasopharynx	IV ($T_2N_3M_0$)	+	CR	§	2	31+
7	M	38	Nasopharynx	IV ($T_4N_0M_0$)	–	PR‡	CR	22+	25+
8	M	45	Nasopharynx	IV ($T_2N_3M_1$)	+	NV	—	—	3
9	M	50	Nasopharynx	IV ($T_2N_2M_1$)	+	NR	—	—	12
10	M	57	Nasopharynx	IV ($T_2N_2M_1$)	+	CR	CR	14+	18+
11	F	16	Nasopharynx	IV ($T_3N_3M_1$)	+	PR‡	—	7+	9+
12	M	46	Nasopharynx	IV ($T_1N_2M_0$)	+	PR‡	CR	6+	9+

*EBV = Epstein-Barr virus, NR = no response, and NV = early death.
†Reduction in tumor volume < 50%.
‡Reduction in tumor volume > 50%.
§Still undergoing CT.

the nasopharynx. As primary treatment, all patients received 25 mg/m^2 of doxorubicin, 10 mg/m^2 of bleomycin, 6 mg/m^2 of vinblastine, and 375 mg/m^2 of dacarbazine, iv, on Days 1 and 14 every 28 days. When it was thought that the maximum clinical response had been achieved (after at least three cycles), RT with ^{60}Co in doses of 4500–6000 rad was delivered to the primary tumor and the bilateral laterocervical lymph node chains. One patient did not receive RT because of an early death, two others because of distant metastases, and one because she is still undergoing CT (table 1).

RESULTS

ABVD as first treatment resulted in CR in four patients; PR with a > 50% reduction of tumor volume in four; PR with a < 50% reduction of tumor volume in one; and no response in two. One patient with extensive disease died 1 day after the first dose of CT and is not included in our evaluable data. Two other patients died: one from pancreatic cancer after 54 months and another from progressive disease after 12 months. Thus, the median survival in the 11 evaluable patients has not been achieved at 28 months. The eight patients who received the combined treatment with ABVD + RT achieved CR. In these patients the median duration of CR has not yet been achieved at 27 months (range, 6–43), and the median survival has not been achieved at 32 months (range, 9+ to 60+) (table 1). Three of the patients in this group had recurrent disease at 10, 34, and 38 months. Toxicity was generally acceptable, with moderate to severe nausea and vomiting during CT and mild mucositis during RT. No important bone marrow toxicity was observed (wbc count nadir, 2900/mm^3; platelet count nadir, 115,000/mm^3). No patient showed doxorubicin cardiotoxicity or the known side effects of bleomycin.

DISCUSSION

Conventional treatment of lymphoepithelioma consists of RT at high energies in doses of 6000–7500 rad to the primary tumor and to the involved laterocervical lymph nodes. With such a treatment the survival rates at 3–5 years in all stages have been reported to vary from 27% to 58% (1–3,13,14). This study was carried out to determine the feasibility and the efficacy of combination CT followed by RT for advanced lymphoepithelioma, in order to improve the local tumor control rate and prevent distant metastases. No important side effects have been observed so far, no patient refused to complete the scheduled cycles of ABVD, and subsequent RT has been well-tolerated. At 27 months the median duration of CR in the patients who received ABVD + RT has not yet been achieved; the present median survival of 32+ months is superior to that reported in the literature for advanced stages of disease (2,3,13,15,16). The combined therapy also permitted the reduction of initial RT doses, with the benefit of less toxicity and the possibility of further RT in local recurrences. The last point must be emphasized in view of the relatively high incidence of recurrences (30%–40%) in previously radically irradiated regions (1–3,13).

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Brief Reports

Phase II Study of AMSA in Patients With Metastatic Breast Cancer: A Cancer and Leukemia Group B Study[1,2]

Philip C. Amrein,[3,*] Morton Coleman,[4] Frederick Richards, II,[5] Ronald F. Poulin,[6] Bernard Bernhardt,[4] Sandra J. Ginsberg,[7] Mark R. Green,[8] Alan Morrison,[9] Engracio Cortes,[10] David Kiang,[11] Vivian Weinberg,[12] and William C. Wood[6]

A new chemotherapeutic agent, AMSA, synthesized by Cain and Atwell (1), has recently passed phase I studies (2,3) and several phase II trials (4,5). It is a member of a new class of antineoplastic agents derived from the three-ring acridine molecule. In phase I studies in man, the dose-limiting toxic effect was myelosuppression, with very little other toxicity (2,3). Marked antitumor activity was seen in acute myelogenous leukemia, with 25%–30% complete remissions in heavily pretreated patients (5). In two previous phase II studies of heavily pretreated patients with breast cancer, the partial remission frequencies were 6% and 15% (4,6). The results of a Cancer and Leukemia Group B study initiated in June 1979 are reported here.

PATIENTS AND METHODS

Patient Selection

Between June 1979 and April 1980, 89 women were entered in this study of AMSA in breast cancer. There were 77 evaluable patients. Twelve patients were disqualified for the following reasons: two received no drug, one received an additional drug, five did not have measurable disease, and four had inadequately transmitted data. All patients were required to have measurable, metastatic, histologically documented breast cancer, wbc count > 4000/μl, platelet count > 100,000/μl, bilirubin < 3.0 mg/dl, and SGOT and alkaline phosphatase less than three times normal. Most patients (88%) had a performance score ≤ 2 (table 1). Nearly all patients were previously treated with two combinations of chemotherapy, usually cyclophosphamide, methotrexate, and 5-FU followed by a doxorubicin-containing combination. Only three patients had not been previously treated with doxorubicin.

Drug Administration

AMSA was initially administered at a dose of 60 or 120 mg/m², depending on the extent of prior chemotherapy and radiotherapy. Treatments were given every 3 weeks, and doses were increased by 20 mg/m² with each treatment until toxicity was encountered or a level of 160 mg/m² was achieved.

RESULTS

Drug Toxicity

Seventy-six patients entered in the study were evaluable for toxicity (table 2). Clearly, the dose-limiting toxic effect was myelosuppression, and wbc counts were more sensitive than platelet counts. Overall, 17% had wbc count nadirs between 1000 and 2000/μl, while 14% had wbc counts < 1000/μl. Thrombocytopenia was less severe; only 10% had a platelet count < 50,000/μl, and no bleeding problems were encountered. Regardless of dose, approximately 20% of the patients experienced some nausea or vomiting, and only 4% reported any sto-

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[1] Received July 13, 1981; revised Nov 9, 1981; accepted Nov 24, 1981.

[2] Supported in part by Public Health Service grants CA-16118, CA-12449, CA-07968, CA-03927, CA-21060, CA-11789, CA-25119, CA-11028, and CA-16450 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Hematology-Oncology Unit, Massachusetts General Hospital, Boston, and the Cancer and Leukemia Group B Operations Office, Scarsdale, NY.

[4] New York Hospital-Cornell Medical Center, NY.

[5] Bowman Gray School of Medicine, Winston-Salem, NC.

[6] Massachusetts General Hospital, Boston.

[7] Upstate Medical Center at Syracuse, NY.

[8] University of San Diego, CA.

[9] Methodist Hospital-SUNY Downstate Medical Center, Brooklyn, NY.

[10] Long Island Jewish Medical Center, New Hyde Park, NY.

[11] University of Minnesota, Minneapolis.

[12] Cancer and Leukemia Group B Operations Office, Scarsdale, NY.

*_Reprint requests to:_ Philip C. Amrein, MD, Hematology-Oncology Unit, Cox 6, Massachusetts General Hospital, 100 Blossom St, Boston, MA 02114.

TABLE 1.—AMSA in breast carcinoma: pretreatment patient characteristics

Characteristic	No. of patients (%)
No. of evaluable patients	77
Median age in yrs (range): 56 (28–74)	
Performance status	
Normal or minor impairment	41 (53)
Bedridden < 50% of time	27 (35)
Bedridden > 50% but < 100% of time	9 (12)
Extent of metastatic disease	
Nodes	33 (43)
Skin	40 (52)
Bone	43 (56)
Visceral	58 (75)
Prior therapy	
Surgery	74 (96)
Combination chemotherapy	77 (100)
Single-drug chemotherapy	33 (43)
Radiotherapy	62 (81)
Hormonal therapy	47 (61)
Immunotherapy	4 (5)
Median time from diagnosis to start of therapy: 48 mos	

TABLE 2.—AMSA in breast carcinoma: toxic effects

Toxic effect	No. of patients (%)
Wbc count nadir (cells/µl)	
> 3000	37 (49)
2000–3000	15 (20)
1000–2000	13 (17)
< 1000	11 (14)
Platelet count nadir (cells/µl)	
> 100,000	57 (75)
50,000–100,000	12 (16)
20,000–50,000	5 (7)
< 20,000	2 (3)
Degree of nausea and vomiting	
None	59 (78)
Mild	8 (11)
Moderate	8 (11)
Severe	1 (1)

matitis. No renal, hepatic, or cardiac toxicity was noted.

Response

Of the 77 patients evaluable for response, there were three (4%) with partial remission (PR) and three (4%) with improvement short of PR. The women with PR were 41, 59, and 61 years old. All had extensive disease with metastases to at least two sites including lymph nodes and skin. All patients had three modalities of prior treatment including a doxorubicin-containing chemotherapy combination. Two PRs occurred at a starting dose of 60 mg/m^2, while one occurred at a starting dose of 120 mg/m^2. Sites of measurable disease response were in skin, nodes, and lung. None of these patients had liver disease, but one had bone metastases and two had lung metastases. Durations of PR were 4, 5, and 15 weeks.

DISCUSSION

This phase II study shows that AMSA in myelosuppressive doses is minimally active in heavily pretreated patients with breast cancer. There was minor gastrointestinal toxicity but no hepatic, renal, or cardiac toxicity. Our results showing 4% PRs and 4% minor regressions are consistent with other phase II studies in advanced, heavily pretreated patients with breast cancer (4,6).

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Phase I Trial of 6-Diazo-5-oxo-L-norleucine (DON) Administered by 5-Day Courses[1,2,3]

Brief Reports

Robert H. Earhart,[4,*] Jim M. Koeller,[4] and Hugh L. Davis[5]

SUMMARY

6-Diazo-5-oxo-L-norleucine (DON), an L-glutamine antagonist, was administered to 25 evaluable patients with refractory advanced solid tumors in a phase I trial. A total of 58 evaluable courses of five daily iv injections every 3–4 weeks were given, at doses ranging from 7.5 to 90 mg/m²/day. The major dose-limiting toxicity was a syndrome of nausea, vomiting, malaise, and anorexia, which became severe at doses > 52.5 mg/m²/day. Diarrhea and stomatitis were less frequent. Hematologic toxicity included mild leukopenia with nadir on Day 6–8 and mild thrombocytopenia with nadir on Day 7–12. Transient decreases in serum calcium to 8.5–8.9 mg/dl were seen in seven of 12 patients receiving ≥ 67.5 mg/m²/day. Dose reduction was required for all patients who received a course of DON at > 67.5 mg/m²/day, and a maximum tolerated total dose of 250 mg/m² (50 mg/m²/day × 5) is suggested for this schedule. Mixed responses were seen in one patient with bladder carcinoma and in one with pulmonary adenocarcinoma.

[Cancer Treat Rep 66:1215–1217, 1982]

Among the array of antibiotics produced by *Streptomyces* fermentation, the diazo analogs of L-glutamine have attracted renewed interest as potential antineoplastic agents (2). These compounds inhibit several biologically important reactions involving L-glutamine, including L-glutamine or L-asparagine incorporation into protein, D-glucosamine biosynthesis, and de novo purine and pyrimidine synthesis (3–6). Although clinical trials were undertaken over two decades ago, interest waned due to unimpressive clinical activity, significant gastrointestinal and mucosal toxicity, supply problems related to technical difficulties with the fermentation process, and the development of other agents with greater apparent potential.

The introduction of the nude mouse xenograft system occasioned testing of a variety of known antineoplastic agents. The L-glutamine antagonists azotomycin and 6-diazo-5-oxo-L-norleucine (DON) displayed activity in xenografted human lung LX-1, colon CX-2, and breast MX-1 systems (2). Review of the clinical data from early trials of DON and careful murine schedule-dependency studies both suggested that further clinical trials of DON should incorporate iv administration of intermittent courses of the drug (7). Accordingly, the Wisconsin Clinical Cancer Center undertook a phase I trial of DON administered by courses of five daily iv injections at 3–4-week intervals.

MATERIALS AND METHODS

Eligibility criteria and evaluation methods were described in one of our previous phase I trial reports (8).

DON was supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD) in sterile 20-ml amber vials of lyophilized powder containing 100 mg of DON, 100 mg of mannitol, sodium hydroxide, and potassium phosphate buffer salts. When reconstituted with 5.0 ml of sodium chloride injection, USP, each milliliter of solution contained 20 mg of DON and 20 mg of mannitol, with sodium hydroxide/0.1 M potassium phosphate buffer at pH 5.4–5.8. The appropriate dose of this solution was diluted in normal saline for injection, USP, to a final concentration ≤ 10 mg/ml and was administered by direct iv injection. Treatments were repeated daily for 5 days every 3–4 weeks, provided

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[1] Received July 17, 1981; revised Oct 26, 1981; accepted Nov 24, 1981.

[2] Supported by contract N01-CM-87208 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Earhart is the recipient of Junior Faculty Clinical Fellowship #388 from the American Cancer Society.

[3] An abstract based on this study has been published previously (1).

[4] Department of Human Oncology, Wisconsin Clinical Cancer Center, Madison.

[5] Division of Hematology/Oncology, Henry Ford Hospital, Dearborn, MI.

**Reprint requests to:* Robert H. Earhart, MD, K4/532 Clinical Sciences Center, 600 Highland Ave, Madison WI 53792.

that patients had recovered from all previous toxicity.

Based on the previous human data that doses of 7.5 mg/m²/day are tolerated for 14–30 days (9), a starting dose of 7.5 mg/m²/day × 5 days was selected. Escalation proceeded in successive groups of three to four patients by a standard modified Fibonacci scheme, using doses of 7.5, 15, 24.75, 37.5, 52.5, 67.5, and 90 mg/m²/day. When significant toxicity was encountered at 90 mg/m²/day, an intermediate level of 75 mg/m²/day was established. Dose escalation within patients was prohibited as a rule, but one patient's dose was escalated from 67.5 to 90 mg/m²/day. Doses were reduced to the next lower level if toxicity was significant in the previous course. When significant toxicity occurred at higher dose levels, further patients were accrued at 52.5 and 67.5 mg/m²/day to develop more quantitative toxicity data near the projected maximum tolerated dose level.

Nausea and emesis were treated with prochlorperazine (10 mg orally or im or 25 mg by rectal suppository). If this failed, chlorpromazine (10–25 mg orally or 25 mg rectally) was used. Prophylactic antiemetics were given in later courses if patients had experienced prior nausea or emesis. Iv fluid replacement was given if clinically indicated.

RESULTS

Twenty-nine patients were entered in this phase I trial. A total of 64 courses of DON were administered, and 58 of these were evaluable. One patient given 15 mg/m²/day and one patient given 67.5 mg/m²/day were unevaluable due to their refusal to complete the initial course of therapy. One patient given 52.5 mg/m²/day and one patient given 67.5 mg/m²/day died 2 weeks after their initial course due to rapidly progressive disease. Two other courses were unevaluable due to inadequate data collection by local hospitals.

Only two patients had not received prior chemotherapy or radiation therapy. One patient had received prior radiotherapy only. The remaining patients had all received prior chemotherapy, usually with more than one agent, and nine of these had also received prior radiation therapy. Nineteen patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and ten had an ECOG performance status of 2 or 3.

Nausea, vomiting, malaise, and anorexia proved to be the dose-limiting toxic effects of DON in this trial. As shown in table 1, the incidence and severity of vomiting, malaise, and anorexia tended to correlate with the dose of DON. Diarrhea and stomatitis occurred sporadically at all dose levels and were not dose-limiting. Although pharyngitis was observed in two patients at higher dose levels, the relationship of this finding of the drug is unclear. Five of 19 treatment courses were terminated early by the patients due to gastrointestinal symptoms, despite vigorous antiemetic therapy. Fluid replacement was required in three courses. Nonhematologic toxicity of DON resulted in significant decreases in performance status in most patients treated at the highest three dose levels. All of these effects were reversible within a few days after treatment.

Hematologic toxicity of DON was relatively mild and never life-threatening. Total wbc count nadirs occurred 6–8 days after initiation of treatment. Both were readily reversible. Transient decreases in serum calcium to 8.5–8.9 mg/dl were observed in seven of 12 patients treated at a dose of ≥ 67.5 mg/m²/day, without concomitant changes in serum albumin. These decreases never produced symptoms of hypocalcemia. Other electrolytes and serum indices of hepatic or renal injury remained within the normal range. Extravasation did not occur, and no phlebitis was observed.

No objective responses meeting standard ECOG criteria were observed in this phase I trial. One patient with bladder carcinoma and one patient with pulmonary adenocarcinoma exhibited mixed responses.

DISCUSSION

Our results and the future development of DON must be evaluated in the light of previous clinical experience. Early trials established that iv or oral doses of DON at doses of 0.1–0.3 mg/kg/day caused glossitis, oral mucositis, and mucosal ulcerations after 7–14 days of treatment (range, 4–20 days). Nausea, vomiting, diarrhea, mild myelosuppression, and azotemia were also seen. Significantly, administration of divided doses at 4- or 6-hour intervals caused more toxicity than did daily doses, which in turn caused more toxicity for a given amount of DON than did treatment every 2–4 days (9). This schedule dependency was mirrored in animal studies, where the single-dose LD50 was > 400 times the LD50 on a daily × 5 schedule. Maximal increases in lifespan were achieved by five or more repeated daily doses in L1210 leukemia (4). Using relatively prolonged courses of daily DON injections, clinical investigations demonstrated single-agent activity in breast, lung, and colon carcinomas, Hodgkin's disease, lymphomas, and choriocarcinoma (2). DON was also reported to lower hypercalcemia associated with

TABLE 1.—Toxicity of DON by dose, type, and grade

Dose (mg/m²/day × 5)	No. of evaluable cases	Grade	No. of courses with —					
			Vomiting*	Stomatitis	Diarrhea	Malaise/anorexia	Leukopenia	Thrombocytopenia
7.5	3	1	1	1	2	2	0	0
		2	1	1	0	0	0	0
15	3	1	1	0	0	1	0	0
		2	2	1	0	0	0	0
24.75	3	1	0	0	3	2	0	0
		2	2	0	0	0	0	0
		3	0	0	0	0	1	0
37.5	3	1	0	1	1	0	1	0
		2	2	1	0	3	0	0
52.5	8	1	0	0	3	0	2	3
		2	7	3	1	6	2	1
		3	1	0	0	1	1	0
67.5	7	1	0	0	2	0	0	0
		2	4	2	0	4	1	1
		3	3	0	0	2	1	0
		4	0	0	0	0	0	1
75	3	1	0	0	1	0	1	1
		2	3	1	0	1	0	1
		3	0	0	0	2	1	0
90	4	1	0	0	3	0	0	1
		2	0	0	0	0	1	0
		3	4	0	0	4	1	0

*Nausea and emesis were graded by ECOG criteria: grade 0, no nausea or emesis; grade 1, nausea without emesis; grade 2, controllable nausea with emesis; grade 3, uncontrollable emesis.

malignant disease but had no effect on normal calcium levels (10), in contrast to our findings.

We conclude that there is a rational basis for further trial of DON on the schedule used in this study, based on animal and early clinical data. A starting dose of 50 mg/m²/day × 5 days every 3–4 weeks is indicated in patients with good performance status. Gastrointestinal toxicity will cause patients to refuse treatment at higher doses in an appreciable percentage of courses.

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Brief Reports

Phase II Study of 4′-Epi-doxorubicin in Metastatic Renal Cancer[1]

S. D. Fosså,[2,*] B. Wik,[3] E. Bae,[3] and H. H. Lien[2]

SUMMARY

In 20 patients with measurable metastatic renal cancer, 4′-epi-doxorubicin (Adriamycin) administration (75 mg/m², every 3 weeks) did not result in any tumor remission. Hematologic and gastrointestinal toxicity was generally mild to moderate. Anthracycline-induced cardiac side effects were not observed in any of the patients.

[Cancer Treat Rep 66:1219–1221, 1982]

4′-Epi-doxorubicin (Adriamycin) (4′-epi-ADR) is a stereoisomer of doxorubicin and its toxicity is reported to be less than that of doxorubicin, particularly with regard to cardiac effects (1). The exact maximum tolerated cumulative dose of 4′-epi-ADR has not been defined but is probably at least 550 mg/m², which is the maximum cumulative dose of doxorubicin.

Preliminary observations have indicated cytostatic effects of 4′-epi-ADR in metastatic renal cancer, even if it is given in moderate single doses (75 mg/m²) (1). A phase II study was therefore performed at the Norwegian Radium Hospital to define the efficacy of 4′-epi-ADR in this malignancy.

PATIENTS AND METHODS

Twenty-one consecutive patients with histologically proven metastatic renal cancer (table 1) were treated with 4′-epi-ADR (75 mg/m² iv, repeated every third week). To avoid any extravasation the drug was given as a bolus injection in a 500-ml iv normal saline infusion. All patients had at least one bidimensionally measurable tumor manifestation.

Response definitions according to the World Health Organization (WHO) were used (2) after at least two treatment courses. 4′-Epi-ADR was discontinued after the second course in cases of progressive disease. If the disease appeared to be stable after two treatment courses, most of the patients received an additional (third) injection of 4′-epi-ADR. If no tumor regression was observed after three courses, 4′-epi-ADR was discontinued in most of the patients. A total of 59 treatment courses were given to all patients.

Hemoglobin, leukocytes, platelets, hematologic liver function, and serum creatinine were analyzed prior to each treatment course. The subjective and objective toxicity of 4′-epi-ADR was evaluated following the recommendations of the WHO (2). An ECG was performed before each 4′-epi-ADR injection and the systolic time interval (STI) was determined at 3-week intervals.

RESULTS

One patient died after the first treatment course due to progressive disease (early death). No tumor remissions were observed after at least two treatment courses in the remaining 20 patients. There was no change in 13 of these patients and disease progression in seven. Twelve patients received three to six courses without reduction of the indicator lesions. There was no change in 11 of these patients and disease progression in one. No change, lasting for 1 and 9 months, respectively, was observed in two of eight patients with indicator lesions which had progressed during the last 2 months before treatment with 4′-epi-ADR.

Table 2 shows the degree of toxicity, which was most often either grade 1 or grade 2 (2). The lowest leukocyte counts (nadir, $1.8 \times 10^3/mm^3$) were observed in patients who had received cytostatic treatment prior to 4′-epi-ADR therapy. The nadir leukocyte count was reached on Days 8–10 of each 3-week cycle. On Day 21, the leukocyte count had usually recovered to normal. Thrombocytopenia (platelets $\leq 100 \times 10^3/mm^3$) was not observed in any case. Anemia (nadir, 7.5 g/100 ml) was most frequent and most severe in patients with wide-

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[1] Received July 31, 1981; revised Oct 13, 1981; accepted Nov 17, 1981.

[2] General Department (*S. D. Fosså*) and Department of Diagnostic Radiology (*H. H. Lien*), The Norwegian Radium Hospital, Oslo.

[3] Department VIII, Cardiovascular Laboratory, Ullevål Hospital, Oslo, Norway.

[*] *Reprint requests to*: S. D. Fosså, MD, General Department, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway.

TABLE 1.—Characteristics of 21 patients with metastatic renal cancer treated with 4′-epi-ADR

Characteristic	No. of patients
Total	21
Age (yrs)	
Mean: 60.1	
Range: 40–70	
Karnofsky index	
Mean: 84.5	
Range: 60–100	
Interval between initial diagnosis and demonstration of metastases (mos)	
Mean: 15.7	
Range: 0–76	
Interval between initial diagnosis and start of 4′-epi-ADR treatment (mos)	
Mean: 23.0	
Range: 1–92	
Nephrectomies	18
Previous cytostatic treatment	8
CCNU (lomustine)-vinblastine	4
Methyl-GAG	3
Ifosfamide	1
Localization of indicator lesions	
Lung	12
Retroperitoneal and/or mediastinal lymph nodes*	5
External lymph nodes	2
Kidney (primary tumor)	2
Cutis/subcutis	1
Os pelvis†	1
Progression of indicator lesions prior to start of 4′-epi-ADR	
Within 2 mos	8
Within 6 mos	1
Within > 6 mos	2
Unchanged within 6 mos	7
Not evaluable	3

*Measurable by computerized tomographic (CT) scan.
†Surrounded by a soft tissue tumor, measurable by CT scan.

spread metastatic disease. No cumulative hematologic toxicity was noted. Alopecia was related with the total cumulative dose of 4′-epi-ADR. After the second course, 19 of 20 patients used a wig.

No major myocardiotoxicity was observed. The STI remained unchanged during 4′-epi-ADR treatment in all patients. However, two patients reported increased angina pectoris after two and three treatment courses, respectively. One of these patients died of myocardial infarction 3 days after the third treatment course. An autopsy was not performed.

DISCUSSION

This study did not confirm previous preliminary results showing anticancer activity of 4′-epi-ADR in renal cancer (1). Objective response was not observed in any of the 20 evaluable patients. The observed subjective and objective side effects were, however, only mild to moderate, so the dose of 4′-epi-ADR might have been too low. Nevertheless, Bonfante et al (1), using the same single doses (75 mg/m^2), observed one remission after the first course in four patients with metastatic renal cancer.

We observed "stabilization" (no change) of indicator lesions in two of eight patients with rapidly progressing disease prior to treatment. In general, such stabilization of any tumor manifestation should not be accepted as response, especially where renal cancer is concerned. Even in untreated patients with metastatic renal cancer, periods of progressive disease may alternate with intervals of stabilization which can last for several months. In phase II studies the efficacy of a cytostatic drug can only be proven by the demonstration of measurable objective remissions (partial response, complete response) in indicator lesions.

The overall acute and subacute toxicity of 4′-epi-ADR seems low when used at the dose given above. It is unlikely that the ischemic heart disease which

TABLE 2.—Acute and subacute toxicity in 59 courses of 4′-epi-ADR (75 mg/m^2 iv, q3wks)

	No. of courses resulting in grade*—			
Toxic effect	0	1	2	3
Anemia	29	12	16	2
Leukopenia	45	7	4	3
Thrombocytopenia	59			
Nausea and vomiting		41	18	
Alopecia		23	26	10
Cardiac arrhythmia	59			
Cardiac dysfunction	59			

*According to Miller et al (2).

affected two of our patients was due to the treatment with 4′-epi-ADR. In general, anthracyclines lead to a primary damage of the myocardium with the clinical symptoms of congestive heart failure (3).

We have no information on the long-term toxicity of 4′-epi-ADR since most of our patients did not receive more than two to four courses. As with doxorubicin, cardiomyopathy may well become a problem when higher accumulated doses of 4′-epi-ADR are used. STI determinations are probably not the best way to demonstrate anthracycline-induced myocardiotoxicity. Biopsies from the myocardium (4) or the assessment of the radionuclide ejection fraction (3) are newer techniques which may be better indicators of anthracycline-induced cardiomyopathy.

CONCLUSIONS

4′-Epi-ADR (75 mg/m^2) does not seem to have cytostatic effects in metastatic renal cancer. When used at the dose given above, the drug seems to be combined with only mild to moderate objective and subjective side effects and may therefore be especially useful for the cytostatic therapy of outpatients when the treatment with anthracyclines is being considered.

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Lack of Efficacy of High-Dose Methotrexate by 30-Hour Infusion in Patients With Progressive Small Cell Carcinoma of the Lung[1]

Brief Reports

Daniel C. Ihde,* Anita Johnston-Early, Desmond N. Carney, Martin H. Cohen, Paul A. Bunn, Francis R. Pelsor, and John D. Minna[2]

SUMMARY

Seventeen patients with small cell carcinoma of the lung (SCCL) refractory to at least one combination chemotherapy regimen were treated with a high-dose methotrexate (HDMTX) program consisting of 1500 mg/m² of MTX by iv infusion at a constant rate over 30 hrs following a priming dose of 50 mg/m² of MTX iv. Administration of calcium leucovorin was begun at the end of the infusion. Treatment was repeated every 2 weeks. No complete or partial responses were observed, and the median time to tumor progression was 4 weeks. Hematologic toxicity was much more severe than anticipated, with five patients having thrombocytopenic bleeding requiring platelet transfusions. This HDMTX regimen was both ineffective and unexpectedly toxic in our heavily pretreated patients with SCCL. Literature review discloses little evidence that HDMTX programs have superior activity to standard doses of MTX in this disease.

[Cancer Treat Rep 66:1223–1225, 1982]

Small cell carcinoma of the lung (SCCL) is highly responsive to initial combination chemotherapy, but few new chemotherapeutic agents investigated in recent years have proven to be efficacious in patients failing intensive drug treatment (1). We have administered high doses of methotrexate (HDMTX) with leucovorin rescue in resistant SCCL because of the relative lack of hematologic toxicity of this regimen, because of the possibility that tumors which progressed during administration of standard doses of MTX might respond to higher dose levels, and because of the reported high response rate to HDMTX in patients with lung cancer (2). A 30-hr infusion program of HDMTX was chosen to allow protracted exposure of tumor cells to concentrations of MTX in the range of 10^{-5} M. This regimen was expected to have reduced potential for renal toxicity in comparison to higher dose, shorter infusion duration programs with higher peak urinary MTX levels (3).

PATIENTS AND METHODS

Seventeen patients (13 males, four females; median age, 55 years) with histologically documented SCCL who had disease progression after at least one combination chemotherapy regimen were treated. All patients had measurable or evaluable tumor lesions and a creatinine clearance > 80 ml/min/1.73 m² of body surface area. Other patient characteristics are shown in table 1. Fourteen patients had previously received MTX in standard doses as part of a combination chemotherapy program. The seven patients with reduced peripheral blood cell counts were treated with the expectation that hematologic toxicity would be modest.

Patients were hydrated with 3 liters/m²/24 hrs of 5% dextrose in water with 40 mEq $NaHCO_3$ and 20 mEq KCl/liter for 12 hrs before initiating HDMTX, during drug therapy, and for 48 hrs thereafter. After a priming dose of 50 mg/m² by iv push, 1500 mg/m² of MTX was infused at a constant dose rate over 30 hrs, with appropriate monitoring of urine volume and pH. At the end of the MTX infusion, calcium leucovorin at a dose of 15 mg/m² was given every 6 hrs × 8 iv and then every 6 hrs × 4 orally. Courses were repeated every 2 weeks (or after toxicity abated) until tumor progression. During and after 25 HDMTX infusions in 12 patients, multiple heparinized plasma samples were obtained for determination of MTX

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[1] Received Aug 21, 1981; revised Nov 4, 1981; accepted Dec 1, 1981.

[2] National Cancer Institute-Veterans Administration Medical Oncology Branch, Division of Cancer Treatment, Veterans Administration Medical Center, Washington, DC.

**Reprint requests to:* D. C. Ihde, MD, National Cancer Institute-Navy Medical Oncology Branch, Bldg 1, National Naval Medical Center, Bethesda, MD 20814.

TABLE 1.—Pretreatment characteristics of patients with SCCL given HDMTX

Characteristics	No. of patients
Ambulatory status*	
Fully	8
> 50%	4
< 50%	3
Bedridden	2
No. of previous chemotherapy regimens	
1	11
2	4
3	2
Tumor stage*	
Limited	3
Extensive	14
Prior radiotherapy	
Yes	14
No	3
Peripheral blood cell counts*	
Adequate†	10
Suppressed	7

*At initiation of HDMTX.

†Wbc count > 4000/µl and platelet count > 100,000/µl.

TABLE 2.—Toxic effects of 30-hr HDMTX infusion in 17 patients

Toxic effects	No. of patients
Maximum leukopenia (cells/µl)	
≥ 4000	4
2500–3900	3
1000–2400	7
< 1000	3
Maximum thrombocytopenia (cells/µl)	
≥ 100,000	5
50,000–99,000	3
25,000–49,000	4
< 25,000	5
Mucositis	6
Esophagitis	1
Diarrhea	2
Severe emesis	1
Elevated liver function tests	4
Dermatitis	1
Elevated serum creatinine	0

concentrations by a competitive enzyme binding assay (4).

Complete or partial response was defined as a 100% or a > 50% decrease, respectively, in the sum of the areas of all tumor lesions, persisting at least 4 weeks. Progressive disease (PD) was defined as a 25% increase in old tumor lesions or the appearance of new lesions.

RESULTS

A median of two HDMTX infusions (range, one to four) was administered. Subsequent infusions could be given only after 3–5 weeks in seven of the ten patients given more than one treatment. No complete or partial response was observed. One patient had a transient decrease in liver metastases before PD was documented. Three patients died without assessment of response. The median time to PD in the remaining 14 patients was 4 weeks (range, 2–8). Median survival from the initiation of HDMTX was 9 weeks (range, 1–29).

Toxicity is shown in table 2. Hematologic toxicity was much greater than anticipated. One patient with leukopenia had documented infection, and five with thrombocytopenia bled, requiring platelet transfusions. Elevated liver function tests were reversible in one patient and persisted in three who had liver metastases or hepatitis B infection. There were no drug-related deaths.

Mean (± 1 SD) plasma MTX levels were 3.57 (± 1.44) × 10^{-5} M, 4.86 (± 1.80) × 10^{-5} M, 4.35 (± 2.93) × 10^{-5} M, 3.10 (± 1.65) × 10^{-5} M, 7.21 (± 8.65) × 10^{-7} M, and 1.85 (± 2.95) × 10^{-7} M at 6, 12, 24, 30, 48, and 72 hrs after the start of the 30-hr infusion. Between ten and 20 determinations were made at each time point. There was no striking relationship between plasma MTX levels and hematologic toxicity. Of three infusions with MTX levels > 1 SD above the mean value at 48 or 72 hrs, only one was associated with severe leukopenia (wbc count < 1000/µl) or thrombocytopenia (platelet count < 25,000/µl). Plasma MTX determinations at 48 and 72 hrs were unremarkable in the two remaining patients with severe hematologic toxicity in whom MTX levels were monitored.

DISCUSSION

HDMTX has been employed in multiple chemotherapeutic regimens for the treatment of SCCL without any clear documentation of its advantage over conventional doses of MTX in this disease (1). Only 30% of treated patients responded to HDMTX used as a single agent in newly diagnosed SCCL, results that are not dissimilar to those anticipated with standard doses of MTX (5). We are unaware of other published data which evaluate the activity of single-agent HDMTX in SCCL. Preliminary results of prospectively randomized (6,7) and sequential (8) studies in patients with SCCL suggest no superiority in response rate or median survival for HDMTX compared to standard doses of the drug when each was incorporated into initial combination chemotherapy programs that included two to five other agents. Similarly, there is little evidence that

HDMTX is of any utility in prophylaxis against central nervous system metastases in SCCL (1,9).

Although our patients were heavily pretreated with both combination chemotherapy and irradiation and in most instances had disease progression in treatment programs containing conventional doses of MTX, no evidence whatsoever of efficacy of HDMTX was encountered in this phase II trial. The hematologic toxicity of the 30-hr infusion of HDMTX in our patients was considerably greater than was reported with a 36-hr infusion using an identical dose, despite comparable mean plasma MTX levels for as long as 72 hrs from the start of therapy (3). This is probably accounted for by the marked differences in prior antineoplastic therapy and pretreatment peripheral blood cell counts in the two patient populations, although the higher dose and different schedule of calcium leucovorin administered by Hande et al (3) could be another possible explanation. Treatment-related toxicity coupled with the lack of antitumor effect discourages the use of HDMTX regimen which we employed in the clinical setting of progressive SCCL.

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Phase I Trial of Pentamethylmelamine[1,2]

Brief Reports

Jaffer A. Ajani,* Fernando F. Cabanillas, and Gerald P. Bodey[3]

SUMMARY

Pentamethylmelamine, an analog of hexamethylmelamine developed for iv use, was administered to 42 patients in a phase I clinical trial. The dose ranged from 0.080 to 2.50 g/m^2/day × 5 and was repeated approximately every 3 weeks. The dose-limiting toxic effects included moderate to severe nausea, vomiting, and central nervous system (CNS) toxicity. Myelosuppression was mild and was not dose-limiting. Antineoplastic activity was observed in four patients. Due to unacceptable gastrointestinal and CNS toxic effects from pentamethylmelamine at the active dose levels, we do not recommend disease-specific phase II trials.

[Cancer Treat Rep 66:1227–1228, 1982]

Pentamethylmelamine (PMM), an analog of hexamethylmelamine (HMM), is ten times more water soluble than HMM and thus can be given iv. In the preliminary study, the antitumor efficacy of PMM appeared similar to that of its parent compound HMM. HMM is active against human bronchogenic, ovarian, breast, and cervical carcinomas (1,2) and lymphomas and sarcomas (3). PMM is more active than HMM against the experimental Colon 38 and P388 tumors. Chemically, PMM is a monodemethylated derivative of HMM and undergoes extensive hepatic metabolism into demethylated compounds (4).

MATERIALS AND METHODS

Our study was open to patients with all types of histologically proven malignancies who had failed standard therapy. Only patients with an estimated survival of 12 weeks were eligible. Patients were required to have a wbc count $> 2000/mm^3$, a platelet count $> 100,000/mm^3$, a serum bilirubin level < 2.0 mg/dl, and a serum creatinine < 2.5 mg/dl. An informed consent was obtained from all patients.

One third of the toxic dose low in dogs was selected as the starting dose; ie, 0.08 g/m^2/day × 5 (based on animal studies that showed slight therapeutic advantage over a single-dose schedule). Required studies included cbc, platelet count (weekly during treatment), liver and renal function tests, lesion measurements, and baseline EEG.

Complete remission was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks, partial remission was defined as a 50% decrease in the size of the measured lesions, stable disease was defined as a steady state of the tumor, and minor response was defined as reduction in the size of the tumor less than that for partial remission for a minimum of 8 weeks.

RESULTS

Forty-two patients were entered in the study. Tumor types included melanoma (12 patients), gastrointestinal cancer (ten), lung cancer (ten), breast cancer (six), ovarian cancer (two), hepatoma (one), and glioblastoma (one). Fourteen patients were evaluable for tumor response. The remaining 28 patients were not evaluable because of lack of measurable lesions in four, inadequate trial due to toxic effects in eight, early death in nine, loss to followup for three, and refusal of further treatment by four. After six 100% dose increases, it became necessary to decrease the dose because of toxicity. Nausea and vomiting were major toxic effects and were dose-limiting in 15 patients. Nausea and vomiting occurred in most patients who received doses ≥ 160 mg/m^2; vomiting became more pronounced at the higher dose levels and peaked during the first 2

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[1] Received Aug 26, 1981; revised Nov 17, 1981; accepted Dec 2, 1981.

[2] Supported by contracts N01-CM-87233 and N01-CM-87185 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Jaffer A. Ajani, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Effect of PMM dose on CNS toxicity

Dose (g/m^2/day × 5)	No. of evaluable courses	No. of patients with CNS toxic effects
0.080–0.64	20	0
1.00–1.50	25	5
2.00–2.50	5	5

TABLE 2.—Forms of CNS toxicity in 10 patients treated with PMM

Signs and symptoms	No. of patients
Disorientation	8
Coma or stupor	5
Irritability	3
Ataxia	2
Visual or auditory symptoms	2
Paresthesias	2
Seizure	1
Loss of taste	1
Weakness	1

days, usually requiring iv hydration and usually resolving by Day 5 or 6 of therapy.

CNS toxicity was not observed with doses < 1 g/m^2 (table 1); it was more severe and frequent with higher doses. Diffuse abnormality of the EEG was commonly observed with clinical CNS toxicity. Ten patients had various forms of CNS toxicity (table 2). Three had metastatic tumor in the brain, and one had superior vena cava syndrome, so the contribution of PMM to CNS toxicity in these four patients is difficult to define. Partial recovery from CNS toxicity occurred in five patients and complete recovery in three; two patients died from progressive peripheral disease.

Myelosuppression was mild and was not dose-limiting, as noted by another study (5). Leukopenia < 1000 cells/mm^3 was not observed in any patient. Thrombocytopenia $< 100,000$ cells/mm^3 was observed in nine patients and $< 50,000$ cells/mm^3 in four patients. With the higher dose levels, greater myelosuppression was observed, but it was still mild. Cumulative toxicity of PMM in the form of myelosuppression was not observed.

Four of 14 patients had less than partial remission, which was of short duration. All responses were seen in the dose range of 1.25–2.5 g/m^2. Responses (one each) were seen in patients with melanoma, hepatoma, ovarian cancer, and breast cancer.

DISCUSSION

This study demonstrates that PMM frequently causes dose-limiting nausea, vomiting, and CNS toxicity, as observed by other investigators (5–8). We observed it frequently at dose levels > 1 g/m^2/day × 5. CNS toxicity occurred frequently and was unacceptable at what appears to be the "therapeutic" range (1.25–2.5 g/m^2) of PMM; all four tumor responses were seen in this dose range. In this study we used 100% dose increases in the absence of dose-limiting toxicity with the previous course. Nausea and vomiting were observed early in the study and were not considered severe enough to interfere with the 100% dose increase, which might have resulted in dose increases up to 2.5 g/m^2/day × 5 that were too rapid. In spite of the dose reduction carried out when CNS toxicity was first encountered, we continue to observe neurologic dysfunction at doses as low as 1 g/m^2/day × 5.

Although PMM appeared to have antineoplastic activity in our study, gastrointestinal and CNS toxic effects were unacceptable. Therefore, we consider PMM too toxic for phase II clinical trials, as also concluded by Goldberg et al (7).

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Phase II Trial of Chlorozotocin in Malignant Melanoma, Breast Cancer, and Other Solid Tumors[1]

Bruce A. Silver, Audrey L. Barlock, Marc E. Lippman, Tom Anderson, and Richard I. Fisher[2,*]

SUMMARY

We have conducted a broad phase II clinical trial of chlorozotocin in 74 patients including 28 with malignant melanoma, 18 with breast cancer, nine with non-Hodgkin's lymphoma, six with nonseminomatous testicular cancer, five with ovarian cancer, four with sarcoma, three with non-beta islet cell carcinoma of the pancreas, and one with anaplastic carcinoma of the thyroid. Objective responses were noted only in 15% of the patients with melanoma and in 11% of the patients with non-Hodgkin's lymphoma. Significant leukopenia and thrombocytopenia were observed only in previously treated patients. Chlorozotocin does not appear to offer clinically significant advantages over other currently available nitrosoureas.

[Cancer Treat Rep 66:1229–1230, 1982]

From 1978 to 1980, the Medicine Branch of the National Cancer Institute conducted a broad phase II clinical trial of chlorozotocin. All 74 patients have now completed chlorozotocin therapy and the results are reported here.

PATIENTS AND METHODS

The histologic diagnoses of the patients have been listed in the Summary. All patients had measurable disease. Patients were required to have a wbc count > 3000/mm^3, a platelet count > 75,000/mm^3, and normal liver and renal function tests (unless the abnormality was due to the malignant process). The patients had a median age of 49 years (range, 18–71), and the male/female ratio was 31/43. Forty-nine patients (66%) had a Karnofsky performance status of 80–100; 21 (28%), 50–70; and four (5%), < 50. Of the 28 patients with melanoma, 13 had had no prior treatment other than surgery and 11 had previously received methyl-CCNU or BCNU (carmustine). Of the remaining 46 patients, all had received prior chemotherapy, but only the three patients with islet cell carcinoma had received a prior nitrosourea, ie, streptozocin. In addition, 14 of the patients with breast cancer had received hormonal therapy.

Chlorozotocin was given as an iv bolus once every 6 weeks at a starting dose of 120 mg/m^2. If no hematopoietic or organ toxicity was observed after two doses, the dose was escalated to a maximum of 150 mg/m^2. Doses were reduced for either nadir or treatment day wbc count and/or platelet count according to a sliding scale. In 140 cycles, the mean dose administered was 94% of the 120-mg/m^2 projected dose. Response was evaluated by standard criteria.

RESULTS

Therapeutic Response

Of the 74 patients, 42 received one cycle, 20 received two cycles, seven received three cycles, two received four cycles, and one each received six, ten, and 13 cycles of chlorozotocin. No responses were observed in patients with breast cancer, nonseminomatous testicular cancer, epithelial or granulosa cell tumors of the ovary, bony or soft tissue sarcoma, non-beta islet cell carcinoma of the pancreas, or anaplastic thyroid cancer. In the 28 patients with malignant melanoma, one complete response (4%) of 5.5 months' duration and three partial responses (11%) of 1.5, 1.5, and 17 months' duration were observed (overall response rate, 15%). The four responding patients had pulmonary nodules, lymph node metastases, subcutaneous local recurrence, and cutaneous local recurrence. No responses were ob-

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[1] Received Aug 31, 1981; revised Dec 1, 1981; accepted Dec 10, 1981.

[2] Medicine Branch, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD.

[*] *Reprint requests to:* Richard I. Fisher, MD, Medicine Branch, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, MD 20205.

served in melanoma patients with liver or other abdominal or pelvic disease. There was one partial response (9%) among 11 patients with melanoma who had received prior nitrosoureas and one complete and two partial responses (18%) among 17 patients without prior nitrosourea exposure. One partial response of 6 months' duration was observed among nine heavily pretreated patients with non-Hodgkin's lymphoma (11%).

Toxicity

Clinically significant myelosuppression was uncommon. Wbc counts < 2000/mm^3 and platelet counts < 50,000/mm^3 occurred only in patients previously treated with chemotherapy. There were no episodes of sepsis or fever requiring antibiotics. Two patients required prophylactic platelet transfusions and two (one with cutaneous bleeding and one with gastrointestinal bleeding) required therapeutic platelet transfusions.

Six patients demonstrated mild, transient hepatic function abnormalities, usually after at least two cycles of therapy; increases in SGOT, SGPT, and/or alkaline phosphatase were 1½–3 times normal. None of these patients had clinical evidence of metastatic disease in the liver; in one patient there was no autopsy evidence of metastatic disease in the liver. Only one patient was symptomatic, with nausea, vomiting, and anorexia. Liver biopsy in this patient revealed a mild increase in the number of hepatocyte mitoses and binucleate hepatocytes with focal areas of single-cell necrosis, compatible with toxic hepatitis. No other significant toxicity was observed.

DISCUSSION

Chlorozotocin appears to have no substantial activity in non-small cell bronchogenic carcinoma, colorectal carcinoma, renal cell carcinoma, or in previously treated breast cancer or soft tissue sarcoma (1–10). Phase II studies appearing only in abstract form have noted the presence of minimal activity in Hodgkin's and non-Hodgkin's lymphomas and melanoma (11–14). Although our own series includes only small numbers of patients with testicular and ovarian cancer, we saw no evidence of activity against these tumors. Chlorozotocin appears to be no more active against melanoma (10%–20% response rate in the literature; 15% in our series) than currently available nitrosoureas. The 9% response rate in our patients with melanoma who had previously received another chloroethyl nitrosourea and the failure of chlorozotocin therapy in three patients with non-beta islet cell carcinoma of the pancreas (streptozocin-resistant) suggest that cross-resistance between these agents is common. It is unlikely that chlorozotocin will have a major impact in any of the malignancies that have been studied to date.

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Phase II Trial of Dianhydrogalactitol in Advanced Renal Cell Carcinoma: A Southwest Oncology Group Study[1,2]

Voravit Ratanatharathorn,[3,*] Laurence H. Baker,[3] Lodovico Balducci,[4] Robert W. Talley,[5] and Barth Hoogstraten[6]

Forty-four patients with advanced renal cell carcinoma (33 males and 11 females; mean age, 57 years) were entered in a phase II study of dianhydrogalactitol (DAG). Twenty-eight patients had had nephrectomy. Sixteen patients had had previous radiation therapy to the involved renal bed or symptomatic metastatic site(s). Twelve patients had been previously treated with cytotoxic drugs, and an additional four patients had received both prior radiation therapy and chemotherapy. An initial dose of 30 mg/m²/day of DAG was administered by iv push daily for 5 days every 4 weeks to good-risk patients, defined as those having normal tolerance to chemotherapy, no prior treatment with nitrosoureas, and normal liver and renal functions (1). The daily dose was reduced to 25 mg/m² for patients who did not meet the previously mentioned criteria. Because of the fatal complications related to myelosuppression encountered during the early phase of this clinical trial, the starting dose was subsequently reduced to 15 mg/m²/day for patients with impaired renal function and/or only one functioning kidney. For patients demonstrating mild or no adverse effects, the dose for subsequent courses was escalated by an increment of 5 mg/m²/day. A similar increment for dose de-escalation was adopted for patients experiencing severe or life-threatening toxic effects. Thus, 21, 7, and 16 patients were treated at a starting dose level of 30, 25, and 15 mg/m², respectively. Patients were considered evaluable for response if they received at least one course of DAG and lived for a minimum of 4 weeks after therapy.

TABLE 1.—Patient characteristics, response, survival, and hematologic toxicity*

Characteristic	Dose level (mg/m²) 30	25	15
No. of patients entered	21	7	16
Male:female	12:9	7:0	14:2
Previous radiation therapy	10	2	8
Previous cytotoxic therapy	6	3	7
Early death	2	1	0
No. evaluable for response	19	6	16
Tumor response (duration)	1 partial (4 wks)	0	0
No. evaluable for toxicity	17	6	15
Drug-related death	3	1	0
Total No. of courses given	48	15	68
Average No. of courses/patient (range)	2 (1–8)	2 (1–8)	4 (1–13)
Median survival (days)	75	105	123 ($P = 0.17$)
No. of escalated dose(s) given (%)	2 (4)	0	8 (12)
No. of de-escalated dose(s) given (%)	2 (25)	0	1 (1)
Hematologic toxicity (% of patients)			
Wbcs × 10³/mm³			
2.0–2.9	5 (29)	0	0
1.0–1.9	0	2 (33)	1 (7)
< 1.0	4 (24)	0	0
Platelets × 10³/mm³			
50–74.9	3 (18)	0	0
25–49.9	1 (6)	3 (50)	0
< 25.0	4 (24)	2 (33)	0

*Unless specified otherwise, values = No. of patients.

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[1] Received Oct 1, 1980; revised Nov 30, 1981; accepted Dec 8, 1981.

[2] Supported in part by Public Health Service grants CA-13392, CA-04919, CA-04915, CA-03096, CA-11233, CA-16957, CA-04920, CA-03389, CA-13238, CA-14028, CA-12213, CA-22411, CA-12644, CA-20319, CA-13612, CA-16388, CA-16385, CA-22416, CA-21116, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Oncology, Wayne State University School of Medicine, Detroit, MI.

[4] Department of Medicine, University of Mississippi, Oxford.

[5] Department of Oncology, Henry Ford Hospital, Detroit, MI.

[6] Department of Medicine, University of Kansas, Kansas City.

*_Reprint requests to:_ Dr. V. Ratanatharathorn (7520), c/o Southwest Oncology Group, Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

Clinical information, hematologic toxicity, and tumor response are summarized in table 1. Of the 41 patients treated at various dose levels, only one brief partial response lasting 4 weeks was observed. Both degree and frequency of myelosuppression were found to be most severe in the group receiving a starting dose of 30 mg/m^2/day. Despite dose de-escalation in 12 subsequent courses (25% of the total number of courses administered), there were three drug-related deaths due to irreversible myelosuppression. Significant myelosuppression was observed only in two patients who received a starting dose of 15 mg/m^2/day, despite dose escalation in eight of the 68 courses of DAG administered. The median survival time of patients receiving the lowest dose level was longer than that of patients in the other two groups; however, the difference is not statistically significant ($P = 0.17$). Other toxic effects noted were confusion and generalized weakness in four patients (three received 15 mg/m^2/day and the other patient received 30 mg/m^2/day).

This relatively large phase II trial of DAG in advanced renal cell carcinoma is clearly adequate to assess drug efficacy in this tumor category. No significant antitumor activity was demonstrated in the groups receiving 30 and 15 mg/m^2/day × 5. Although the number of patients treated with 25 mg/m^2/day × 5 was small (six patients), none showed any response. It is possible that the dose level of 15 mg/m^2/day may not be sufficient to show antitumor effect in view of the minimal degree of myelotoxicity observed. Nonetheless, no clinical benefit could be demonstrated at the highest dose level which produced significant myelotoxicity. In fact, our data suggest that a dose of 30 mg/m^2/day may even adversely influence patients' survival. While it can be reasonably argued that previous therapy with cytotoxic drugs, especially alkylating agents, might preclude patient response to DAG, our data do not support this, since the majority of patients were not previously treated with cytotoxic drug(s). We therefore conclude that DAG has no role in the therapy for advanced renal cell carcinoma.

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Hexamethylmelamine-CAF (Cyclophosphamide, Methotrexate, and 5-FU) and Cisplatin-CAF in Refractory Ovarian Cancer[1]

Clinical Trials Summaries

C. Sessa,[2] M. D'Incalci,[3,*] I. Valente,[2] G. Bolis,[4] N. Colombo,[2] and C. Mangioni[2,5]

Fifty-three patients (19–73 years of age) with epithelial ovarian cancer not responding to or relapsing while receiving PAC (cispiatin, doxorubicin, and cyclophosphamide) or HAC (hexamethylmelamine [HMM], doxorubicin, and cyclophosphamide) regimens (1) received either the combination of cyclophosphamide at a dose of 100 mg orally daily on Days 1–14; methotrexate (MTX), 25 mg/m^2 iv on Days 1 and 8; 5-FU, 300 mg/m^2 iv on Days 1 and 8; and HMM, 150 mg/m^2 orally daily on Days 1–14 (HMM-CAF), or the above combination with cisplatin at a dose of 50 mg/m^2 iv on Day 1 as a 1-hour infusion (cisplatin-CAF) in place of HMM. Treatment was repeated every 4 weeks. If the wbc count decreased to $< 3000/\mu l$ or the platelet count to $< 100{,}000/\mu l$, treatment was deferred until the counts increased above these values. The night before administration of cisplatin, patients received 1 liter of saline and 1 liter of 5% glucose containing 40 mEq of KCl and 10 IU of insulin, to maintain diuresis of 200 ml/hour. If urine output decreased to < 100 ml/hour and was not modified by a subsequent increase in fluids, patients received 12.5 g of mannitol by iv push. A complete physical examination and laboratory tests were done before each cycle.

A partial response was defined as a > 50% reduction in the sum of tumor diameters for at least 1 month; no change was defined as a < 50% reduction or a < 25% increase in the sum of tumor diameters for at least 1 month; and disease progression was defined as a > 25% increase in the sum of tumor diameters or the appearance of new tumors. The same criteria were applied when response was evaluated by peritoneoscopy (ie, when the known residual tumor was not clinically assessable or not exactly quantifiable by pelvic examination alone).

Table 1 shows the various responses with HMM-CAF in 18 patients. The median number of treatment cycles was two. Only one patient (5%) achieved a partial response, confirmed by laparoscopy, lasting 8 months. Moderate nausea and vomiting were seen in 45% of the patients after MTX and 5-FU administration. Forty-four percent of the patients had stomatitis. Leukopenia, defined as a wbc count $< 3000/\mu l$, occurred in 50% of the patients.

Table 1 also shows the various responses with cisplatin-CAF in 35 patients. The median responses lasted 7, 7, 10, 11, and 19 months. The median survival was 14 months (range, 11–27) for responders and 6.5 months (range, 2–23) for nonresponders.

After cisplatin infusion all patients had severe nausea and vomiting refractory to low doses (0.2 mg/kg) of metoclopramide. Thirty-two percent of the patients had moderate nausea and vomiting after MTX and 5-FU injections on Day 8 of treatment. Mucositis was seen in 54% of the patients, leukopenia was seen in 20%, and anemia, defined as a hemoglobin decrease > 2 g/100 ml, was seen in 29%. A mild increase in serum creatinine (30%–40%) was observed in 17% of the patients, without ever reaching pathologic values. This low nephrotoxicity may be due to the hydration provided at the same time as cisplatin and MTX.

This study confirms the inefficiency of HMM-CAF as second-line chemotherapy (2); cisplatin-CAF is also not recommended in patients previously treated with multidrug therapy because the very low response is outweighed by severe toxicity.

In general, these conclusions agree with previous failures reported as second-line chemotherapy for ovarian cancer (3,4).

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[1] Received Aug 4, 1981; revised Nov 25, 1981; accepted Dec 8, 1981.

[2] I Clinica Ostetrica-Ginecologica, Università di Milano, Italy.

[3] Istituto di Ricerche Farmacologiche "Mario Negri," Milano, Italy.

[4] IV Clinica Ostetrica-Ginecologica, Università di Milano, Italy.

[5] We acknowledge the contribution of the Italian Association for Cancer Research, Milan, Italy.

*_Reprint requests to_: M. D'Incalci, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milano, Italy.

TABLE 1.—Response to HMM-CAF and cisplatin-CAF in 53 patients with ovarian cancer

Previous chemotherapy	Treatment	No of patients	Response		
			Partial	No change	Disease progression
PAC	HMM-CAF	18	1	2	15
HAC	Cisplatin-CAF	35	5	2	28

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Phase II Study of AMSA, Prednisone, and Chlorambucil (APC) in the Treatment of Refractory Breast Cancer: A Northern California Oncology Group Study[1,2]

Michael A. Friedman,[3,*] Fred Marcus,[4] Marsha Kohler,[5] and Kenneth J. Resser[3,6]

Single-agent doxorubicin and doxorubicin-containing combinations of drugs have been used for patients whose breast cancer has been unresponsive to primary chemotherapy or whose disease has progressed after remission (1–3). However, no single agent or combination of drugs has proven to be effective. It has recently been reported that patients with advanced refractory breast cancer have had responses to AMSA, presumably a DNA intercalator. An initial phase II study of patients with breast cancer (4) indicated that four of 26 patients responded to AMSA at a dose of 40 mg/m²/day × 3 every 3 weeks. Recognizing that AMSA (or any single agent) alone was unlikely to result in profound therapeutic benefit, we combined it with chlorambucil and prednisone (APC)—the former because preclinical and clinical data suggested non-cross-resistance with cyclophosphamide (H. Skipper and K. Kohn, personal communication) and the latter because of its minimal toxic effects. We recognized, of course, that this phase II trial was not strictly a test of AMSA, but rather of this drug combination.

From May to December 1980, 30 patients with presumed incurable breast cancer gave informed consent and were entered in the Northern California Oncology Group 1B92 protocol study (table 1). All patients had failed a previous 5-FU-doxorubicin program and 22 had failed a previous cyclophosphamide-methotrexate-5-FU program. Twenty-five patients had had previous radiation therapy and 22 had received previous hormonal therapy. High-risk patients were defined as those who had Karnofsky performance scores $\leq 50\%$, previous combination chemotherapy for ≥ 1 year, previous extensive radiation therapy (exposure of $> 50\%$ of bone marrow), or bilirubin levels ≥ 2 mg/100 ml.

Low-risk patients received AMSA (120 mg/m²) iv on Day 1 and daily oral doses of prednisone (40 mg/m²) and chlorambucil (0.2 mg/kg) on Days 1–7. High-risk patients received the same amount of prednisone and chlorambucil but only 90 mg/m² of AMSA. Both groups repeated this therapy on Day 22. Modification of this dose schedule was based on hematologic toxicity.

The criteria for response were similar to those of Hayward et al (5).

None of the patients achieved an objective response. Stable disease was noted in six low-risk and in nine high-risk patients. Median survival was 64 days for the low-risk group and 52 days for the high-risk group. As expected, the median survival of patients whose disease stabilized was longer than that of patients with progressive disease (115 vs 40 days).

Hematologic toxicity was the most important side effect of this treatment. Approximately two thirds of all patients had a wbc count $< 3000/\text{mm}^3$. Thrombocytopenia was less severe and less frequent. Nausea and vomiting, for the most part, were infrequent and mild.

This phase I study of APC is not a precise test of the effectiveness of AMSA, but rather of this combination, which includes AMSA. The dose of

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[1] Received Aug 17, 1981; revised Nov 2, 1981; accepted Dec 8, 1981.

[2] Supported by Public Health Service grant CA-21744 (in part by the Northern California Oncology Group Study) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Institutions participating in this study were the University of California at San Francisco; the University of California at Davis; Greater Sacramento Cancer Council; Kaiser Hospitals in San Francisco, South San Francisco, and Redwood City; David Grant Medical Center; South Bay Oncology Program; and Mount Zion Hospital.

[3] Cancer Research Institute, University of California, San Francisco.

[4] Redwood Medical Clinic, Redwood City, CA.

[5] Northern California Oncology Group, Palo Alto.

[6] We acknowledge Lisa Gerretson and Paulette De Hass-Harper for their assistance in providing quality control and data analysis.

**Reprint requests to:* Michael A. Friedman, MD, Cancer Research Institute, 1282-M, University of California, San Francisco, CA 94143.

Table 1.—APC therapy: patient characteristics and toxic effects

	Low-risk patients	High-risk patients
No. of patients entered	9	21
Mean age in yrs (range)	61 (52–72)	53.6 (36–75)
No. of patients with—		
Karnofsky performance status (%)		
80–100	4	9
60–70	5	4
30–50	0	8
Menopausal status		
Premenopausal	1	6
Perimenopausal	1	2
Postmenopausal	7	13
Sites of metastasis		
Soft tissue	5	10
Lung	4	11
Liver	6	7
Bone	4	16
Brain	0	1
Median (range)		
Duration of previous chemotherapy in mos	7 (1–11)	17 (10–41)
Interval between end of previous chemotherapy and start of APC in mos	1 (0–4)	1 (0–6.5)
No. of APC cycles received	2 (1–7)	2 (1–4)
Most severe hematologic toxic effects		
Wbc count (cells × 10^3/mm^3)		
2.0–3.0	1	7
1.0–2.0	4	3
< 1.0	1	4
Platelet count (cells × 10^3/mm^3)		
50–90	2	6
25–50	1	0
< 25	1	2

AMSA used in our study, however, was the same as that used in the single-agent study of Legha et al (4).

Preliminary phase I–II data of AMSA seemed to indicate a promising response rate. This hope, however, was not borne out in this group of patients. Based on its lack of efficacy, we cannot recommend this combination for patients with advanced breast cancer.

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Clinical
Trials
Summaries

Phase II Evaluation of AMSA in Patients With Stage III–IV Malignant Melanoma[1,2]

Joachim Z. Fuks,[3,*] David A. Van Echo,[3] Joseph Aisner,[3] Harvey Schipper,[4] Martin Levitt,[4] and Peter H. Wiernik[3]

AMSA was selected for clinical trials because evidence of antitumor activity had been noted in animal solid tumor model systems and in phase I studies (1–3).

Sixteen patients with measurable, histologically proven, malignant melanoma who had failed surgery or prior antitumor therapy were given AMSA as a single iv infusion, with treatments repeated every 21 days. The initial dose was 150 mg/m^2, with subsequent doses being adjusted according to the severity of toxicity on Day 21. If no toxic effects were recorded on Day 21, subsequent doses were escalated by increments of 30 mg/m^2. Therapy was continued until there was evidence of disease progression.

Characteristics of the 16 treated patients are shown in table 1.

All patients were evaluable for response and toxicity. No objective responses were seen. Five patients had rapidly progressive disease after the first AMSA course. Calculated from the first day of treatment, 11 patients had initial disease stabilization (6–18 weeks) and then disease progression.

Toxicity data are based on the 34 fully evaluable courses in all 16 patients. Of these patients, five received one course, eight received two courses, and one each received three, four, and six courses. Two patients received three courses of AMSA at an escalated dose of 180 mg/m^2. The maximal dose of AMSA given in this study was 210 mg/m^2 for one course. The major toxic effect was leukopenia (table 1). No toxic deaths were noted. One patient developed *Staphylococcus aureus* bacteremia associated with neutropenia. No clinically apparent cardiac, renal, or hepatic dysfunction was noted.

Table 1.—Patient characteristics and drug toxicity of AMSA in malignant melanoma

Characteristic	No. of patients
Entered/evaluable	16/16
Median age in yrs (range): 52 (24–66)	
Male/female	13/3
Previous treatment	
Chemotherapy (CT)	6
CT + immunotherapy/CT + radiotherapy	1/1
Immunotherapy	2
Radiotherapy	2
Metastatic site(s)	
Cutaneous only	1
Lymph nodes only	3
Cutaneous and lymph nodes	2
Brain	2
Visceral	10
Bone	3
Toxic effects	
Wbc count nadir (cells/μl)	
300	1
301–999	0
1000–1499	3
1500–2999	11
Platelet count nadir (cells/μl)	
<100,000	1
Nausea	3
Chemical phlebitis	1

Although 12 of the 16 patients had received prior therapy, the performance status of all patients was good. In this study, no objective response precludes a 20% level of activity, with 95% confidence limits. Thus, AMSA does not have sufficient antitumor activity to warrant further investigation in patients with advanced malignant melanoma.

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[1] Received Aug 18, 1981; revised Dec 21, 1981; accepted Jan 5, 1982.

[2] Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Baltimore Cancer Research Center, University of Maryland Hospital.

[4] Manitoba Cancer Research Foundation, University of Manitoba, Winnipeg, Canada.

[*] *Reprint requests to:* Joachim Z. Fuks, MD, Baltimore Cancer Research Center, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

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Phase II Study of High-Dose Megestrol Acetate in Patients With Advanced Malignant Melanoma[1]

Edward T. Creagan,* Alan J. Schutt, David L. Ahmann, and Stephanie J. Green,[2,3]

Additive hormonal modalities may influence the clinical course of malignant melanoma. An intriguing report noted objective responses from 6α-methylpregn-4-ene-3,11,20-trione, a synthetic pregnenetrione, in five of 44 patients (1). A related compound, medroxyprogesterone acetate, achieved a 22% response rate in patients who had previously failed conventional chemotherapy (2), a group traditionally refractory to subsequent systemic treatment. We report here a phase II study of high-dose megestrol acetate in patients with disseminated melanoma, most of whom had failed previous chemotherapy.

MATERIALS AND METHODS

Twenty ambulatory outpatients with measurable, biopsy-proven, advanced malignant melanoma received megestrol acetate at a dose of 250 mg orally twice a day until death or evidence of tumor progression (table 1). All patients except two had received prior chemotherapy; two had had prior hormonal therapy with tamoxifen and had failed that program.

Evaluation

Prior to starting treatment and before subsequent monthly reassessments, patients underwent complete medical evaluation, chest X-ray, and routine hematologic and chemical parameters.

Objective regressions were defined using traditional criteria: *complete response*—the absence of all measurable disease; *partial response*—a ≥ 50% decrease in the product of the longest perpendicular diameters of the most clearly measurable indicator lesions without subjective deterioration; and *progressive disease*—a > 25% increase in the product of any indicator lesion or the appearance of new lesions. We calculated "time to progression" from the first day of treatment to date of progression or last followup.

RESULTS

None of the patients satisfied the stated criteria for an objective response. Two thirds of the study participants demonstrated disease progression at the time of their initial monthly reassessment. The median times to progression and survival were 1 and 4 months, respectively, by the Kaplan-Meier method. There was little subjective or objective toxicity from this regimen.

DISCUSSION

Utilizing the dose and schedule of megestrol acetate chosen for this study, we were unable to achieve any meaningful reprieve for our patients with advanced malignant melanoma. Most patients had visceral involvement and had received prior chemotherapy, prognostic covariates which usually portend a dismal result from subsequent systemic treatment. However, even for this patient population we would anticipate a meaningfully active drug to produce a response probability of ≥ 0.15, and our results are inconsistent with a response probability this high ($P = 0.05$).

Our findings disagree with other studies of hormone therapy in patients with malignant melanoma in which responses for tamoxifen, diethylstilbestrol, and progestins have been reported (2–4). The therapeutic implications of these reports are tenuous, especially in view of the notoriously unpredictable clinical course of melanoma. Nevertheless, there may be subsets of patients with melanoma for whom hormonal treatments would be appropriate as evidenced by striking and durable regressions (1). The occurrence of estrogen receptor protein (ERP) in some patients with melanoma was a promising therapeutic discriminant. Unfortunately, there has been

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[1] Received Sept 30, 1981; revised Nov 23, 1981; accepted Jan 5, 1982.

[2] Division of Medical Oncology (*E. T. Creagan, A. J. Schutt,* and *D. L. Ahmann*), and Cancer Statistics Unit (*S. J. Green*), Mayo Clinic and Mayo Foundation, Rochester, MN.

[3] We thank Mead Johnson Pharmaceutical Division, Evansville, IN, for supplying the drug used in this study.

**Reprint requests to.* E. T. Creagan, MD, Mayo Clinic, Rochester, MN 55905.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Male	8
Female	12
Median age in yrs: 52.5	
Performance score	
0,1	12
2,3	8
Dominant disease	
Visceral	14
Soft tissue	6

little correlation between ERP and response to additive hormonal programs (4). Moreover, recent investigations have detected nonspecific protein binding in melanoma biopsy specimens, which seriously challenges the validity of previous ERP data in malignant melanoma (5).

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Inadvertent Overdose With Lomustine (CCNU) Followed by Hematologic Recovery

Hematopoietic toxicity is the dose-limiting factor in cancer therapy with lomustine (CCNU) (1,2). Due to its prolonged myelotoxicity, therapy is generally limited to a dosage of 100–130 mg/m^2 every 6–8 weeks. A correlation between the severity of leukopenia and thrombocytopenia and tumor response has been demonstrated for lomustine (2), suggesting that a beneficial tumoricidal effect might be obtained with megadose lomustine. One approach to this problem might be infusing autologous bone marrow after high-dose lomustine.

A patient with colorectal carcinoma metastatic to the liver and lung was treated with lomustine. Over the course of 1 week, he mistakenly took a prescription intended for use during four cycles. The total dose was 1120 mg (12.5 mg/kg, or 550 mg/m^2). The patient developed severe bone marrow aplasia. With supportive therapy, but without the benefit of autologous marrow infusion, his bone marrow recovered. There was no objective reduction in the size of his tumor.

CASE REPORT

A 59-year-old white male was treated in April 1976 with an abdominoperineal resection for a carcinoma of the rectum. Lymph nodes were noted to contain carcinoma. Extensive staging procedures were negative, and the patient was classified as having a Dukes C adenocarcinoma of the rectum. For 1 year after surgery the patient received adjuvant 5-FU chemotherapy (10 mg/kg/week iv).

The patient remained asymptomatic without evidence of recurrence for approximately 3 years. His carcinoembryonic antigen (CEA) after surgery was 3 ng/ml and remained near that level until May 1979 when it increased to 17 ng/ml. Blood chemistries, liver-spleen scan, and chest X-ray remained normal. The CEA increased over the next 6 months, and by January 1980, it was 108 ng/ml, while his liver function tests remained normal (SGOT, 37 IU/liter; alkaline phosphatase, 75 units/liter; and bilirubin, 0.5 mg/dl). At this time, multiple small metastases were detected on liver scan, and a left lower lobe pulmonary nodule was seen on chest X-ray. The decision was made to begin lomustine chemotherapy at 130 mg/m^2. The first dose was 160 mg (⅔ dose) and the patient was to return in 6 weeks for a repeat cbc and a second dose. He was instructed to take four tablets (40 mg/tablet) at bedtime once only and was given a prescription for thirty 40-mg tablets of lomustine. The patient did not fill the prescription for 3 weeks. By the time he finally began the lomustine, he had forgotten the physician's instructions in regard to one dose only. He proceeded to take four tablets at bedtime each night, until all but two of the tablets were gone. The total dose was 1120 mg. He had minimal nausea with the medication but no other early toxic effects. Two weeks later he began to notice lethargy and anorexia, easy bruisability, and a petechial rash on his arms and legs.

The patient was immediately hospitalized. His platelet count on admission was 1.1 × 10^9/liter, wbc count was 2.6 × 10^9/liter, and hemoglobin was 13 g/dl with 0.1% reticulocytes. The wbc count decreased rapidly and by the 6th hospital day, the granulocyte count was 0. The patient was placed in reverse isolation and supported with platelet and rbc transfusions. His hospital course was complicated by sepsis, which was treated with amikacin and carbenicillin. Urinalysis and creatinine were normal throughout the hospitalization, suggesting no renal toxicity. After approximately 3 weeks the patient was discharged and sent home with hemoglobin of 10 g/dl with 1.7% reticulocytes, wbc count of 3.4 × 10^9/liter with > 50% granulocytes and monocytes, and platelet count of 85 × 10^9/liter. His CEA increased to 200 ng/ml during hospitalization, and the chest X-ray and liver scan remained unchanged. During hospitalization, liver enzymes began to increase (SGOT, 81 IU/liter; SGPT, 165 IU/liter; and alkaline phosphatase, 96 units/liter). Hepatitis B antigen was negative, and this enzyme elevation was interpreted as posttransfusion non-A, non-B hepatitis. Liver enzymes and bilirubin reached a peak 2 months after discharge (SGOT > 300 IU/liter; alkaline phosphatase > 350 units/liter; and bilirubin, 8.2 mg/dl) and began to return to normal by 5 months after discharge (SGOT, 182 IU/liter; alkaline phosphatase, 147 units/liter; and bilirubin, 1.6 mg/dl). There was no change in the status of the tumor during this time. Twenty-two months later the CEA was 216 ng/ml; liver function tests were normal except for a SGOT of 91 IU/liter; liver-spleen scan and chest X-ray were unchanged; and all blood cell counts were normal.

DISCUSSION

This case illustrates the hazards of prescribing orally active cytotoxic agents to patients for use at home. Despite a discussion with the patient outlining the toxicity of the drug and the schedule (one dose every 6–8 weeks), the patient still took all of the medication over 7 days.

This case also illustrates two other points. First, a total dose of 550 mg/m^2 (12.5 mg/kg) of lomustine is not necessarily a lethal myelotoxic dose. The lethal myelotoxic dose of lomustine is not established in man. Studies in dogs have demonstrated that lethal myelotoxicity developed at a dose of 10 mg/kg in three of four dogs and at a dose of 15 mg/kg in all of five dogs (3). These animals were supported with antibiotics and platelets but died from sepsis and bleeding within 10 days. The possibility of eventual bone marrow recovery if they had survived longer is unevaluable. Our patient's dose of lomustine was 12.5 mg/kg, or 550 mg/m^2, with full hematologic recovery. Other patients given similar or higher doses of nitrosoureas have had the benefit of autologous bone marrow rescue. In those cases the lethal myelotoxic dose was unevaluable.

The second point is that there was no objective decrease in the size of the tumor despite this large dose of lomustine. A highly significant association between the severity of leukopenia and thrombocytopenia and tumor response to lomustine has been reported (3). This has led investigators to consider treatment with megadose lomustine and "rescuing" the patient with previously cryopreserved autologous bone marrow. In spite of our patient's very high dose of lomustine, there was no objective response. His pulmonary nodule and liver scan remained unchanged. The increase in CEA was difficult to interpret in light of the patient's hepatitis, because hepatitis alone produces an increase in CEA (4). Data from this patient suggest that, although increasing the dose may correlate with response of sensitive tumors, increasing the dose and toxicity may not have a major effect against resistant tumors. We cannot conclude, however, that there was no beneficial effect, because there was no objective progression of the tumor 22 months following therapy.

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Kenneth A. Foon*
Charles M. Haskell

Research and Medical Services
Wadsworth Veterans Administration
Medical Center and
Department of Medicine
UCLA School of Medicine
Los Angeles, CA 90024

January 28, 1981

**Reprint requests to:* Kenneth A. Foon, MD, Biological Response Modifiers Program, NCI-Frederick Cancer Research Facility, Bldg 560, Frederick, MD 21701.

Phase II Study of AMSA in Patients With Advanced Ovarian Cancer

AMSA is one of the many acridine derivatives prepared by Cain et al (1) that have been shown to have significant antitumor activity in a number of animal tumor systems (2). Results of phase II trials with AMSA indicate only modest antitumor efficacy in a number of solid tumors (3).

Twenty patients with histologically proven advanced ovarian adenocarcinoma were entered in this study. Each patient had an Eastern Cooperative Oncology Group performance status ≥ 2 (median, 2), had received no chemotherapy in the previous 3 weeks, and had baseline hemoglobin > 10 g/liter, wbc count $> 3 \times 10^9$/liter, platelet count $> 100 \times 10^9$/liter, plasma creatinine < 1.5 mg/100 ml, and plasma bilirubin < 2 mg/100 ml. Good-risk patients were those with age ≤ 65 years and normal liver function tests (serum bilirubin, alkaline phosphatase, and transaminases) and without prior extensive radiotherapy or hematologic intolerance of chemotherapy. Poor-risk patients and the one very poor-risk patient were > 65 years old and had received extensive prior radiotherapy or were hematologically intolerant of chemotherapy. Poor-risk patients differed from the very poor-risk patient only in the degree of liver function impairment (2–3 × normal versus > 3 × normal). Patients were 41–78 years old (median, 55) and only two were > 70 years. Fourteen patients were evaluable for tumor response, and six with nonmeasurable disease were evaluable for toxicity only. All patients had received previous chemotherapy including alkylating agents, and 16 (80%) had responded; 15

TABLE 1.—Patient characteristics

Characteristic	No. of patients/ No. evaluable
No. of patients entered: 20	
Median age in yrs (range): 55 (41–78)	
Prior treatment	
Chemotherapy	
Cisplatin	17/12
Chlorambucil	13/9
Cyclophosphamide	6/5
Doxorubicin	6/5
5-FU	5/5
Mitomycin	4/2
Methotrexate	3/3
Hexamethylmelamine	3/3
Vincristine	1/0
Surgery	15/10
Radiotherapy	4/3

had undergone prior surgery (11 radical and debulking) and four had had prior radiotherapy (table 1).

AMSA was administered iv as a 60-minute infusion in 500 ml of 5% dextrose every 21 days. Fifteen good-risk patients received a dose of 120 mg/m^2, four poor-risk patients received a dose of 90 mg/m^2, and one very poor-risk patient received a dose of 60 mg/m^2. If there was no substantial myelosuppression, subsequent doses were increased by 30 mg/m^2 until myelosuppression or progressive disease occurred. Significant myelosuppression necessitated dose reduction by 30 mg/m^2. A single AMSA course with 3 weeks' followup was regarded as adequate for evaluating toxicity, but two treatments were required for assessment of tumor response. Standard criteria for tumor response were employed (4).

RESULTS AND SUMMARY

The median number of courses was two: five patients received one course, eight had two courses, two had three courses, two had four courses, and one each had five, seven, and nine courses.

Two of the 14 patients evaluable for response demonstrated partial tumor response lasting 9 and 18 weeks, respectively. In eight patients, tumor remained stable for 3–24 weeks (median, 12), and in four, disease progressed rapidly.

Myelosuppression was dose-limiting; the median wbc count nadir was 2.1×10^9/liter (range, 0.5–3.9), and the median platelet count nadir was 160×10^9/liter (range, 31–445), both occurring in Week 2 after treatment. In eight patients, the hemoglobin nadir decreased > 20 g/liter from the pretreatment level. The decrease in hemoglobin was independent of the AMSA dose, but investigations to exclude hemolysis proved inconclusive.

Five patients developed moderate nausea and vomiting with doses of AMSA $\geq$ 120 mg/m^2. This was confined to the first two courses in two patients but occurred in all courses in the other three. There was no renal, hepatic, neurologic, mucocutaneous, pulmonary, or cardiovascular toxicity observed.

In six patients, dose escalation was possible: to 180 mg/m^2 in one good-risk patient and 150 mg/m^2 in two; to 120 mg/m^2 in two-poor-risk patients; and to 90 mg/m^2 in the very poor-risk patient. Dose reduction was required (on hematologic grounds) in four good-risk patients and in one poor-risk patient.

In summary, after treatment with AMSA, we observed only two partial responses among 14 patients, both in patients who had received $\geq$ 120 mg/m^2 of AMSA. The present study confirms that the initial dose for good-risk patients should be 120 mg/m^2, as recommended by several phase I studies (5). Only 20% of the good-risk patients tolerated dose escalation. We conclude that AMSA has modest antitumor efficacy as a single agent in previously treated patients with advanced ovarian cancer.

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John H. Kearsley
Alan S. Coates
Richard M. Fox
Martin H. N. Tattersall*

Ludwig Institute for Cancer Research
(Sydney Branch)
Blackburn Bldg
University of Sydney
Sydney, NSW 2006, Australia

Jonathon Page
John A. Levi

Department of Medical Oncology
The Royal North Shore Hospital
St. Leonards, NSW 2065, Australia

November 16, 1981

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*To whom reprint requests should be sent.

Mitomycin-Induced Hemolytic Uremic Syndrome: Case Presentation and Review of Literature

Mitomycin (mitomycin C; MM-C) is a potent alkylating agent. It is active against a variety of human neoplasms. The main undesirable effects of MM-C include myelosuppression, GI toxicity, skin rash, alopecia, and pulmonary fibrosis. It has a nephrotoxic potential, but the true incidence and histopathologic characteristics of this toxic effect remain to be verified (1). More recently, microangiopathic hemolytic anemia (MAHA) with progressive renal failure and cardiopulmonary decompensation were described in patients receiving MM-C (2–6). We report here on a patient with breast cancer who received MM-C and tamoxifen (TAM) and developed similar clinical and laboratory findings.

CASE REPORT

A 55-year-old woman underwent a bilateral mastectomy for a bilateral infiltrating ductal carcinoma of the breasts in January and August 1974, followed by irradiation to the regional lymph nodes. Since August 1975, she was treated with a variety of chemotherapeutic agents including methotrexate, melphalan, doxorubicin, cyclophosphamide, and vincristine. A total of 450 mg/m^2 of doxorubicin was given. In July 1979, a biopsy-proven lymph node recurrence was noted. MM-C (12 mg/m^2 iv) every 5–6 weeks and TAM (10 mg orally) twice a day were started. The patient received a total of 60 mg/m^2 of MM-C from August 10, 1979, to January 15, 1980, resulting in the complete disappearance of the cervical lymph node. On August 6, 1979, the hemoglobin (Hgb) was 12 g/dl and the platelet count was 248,000/mm^3. On October 30, 1979, after two courses of MM-C, the Hgb was 9.6 g/dl, and the platelet count was 175,000/mm^3 (fig 1). The patient complained of exertional dyspnea and peculiar spasms in the hands, calves, and feet; she had no fever and no neurologic symptoms. The anemia progressed and the peripheral smear and laboratory tests showed hemolytic microangiopathy. The platelet count was 49,000/mm^3 and Coombs' tests were negative. In December 1979, serum creatinine was 1.0 mg/dl and Hgb was 7.0 g/dl; repeated rbc transfusions were given. Following each transfusion, Hgb decreased to the pretransfusion level. In March 1980, serum creatinine was 1.7 mg/dl; haptoglobin, 2.0 mg/dl; methemalbumin, 7.7 mg/dl (normal = 0.2–3.6); reticulocyte count, 3.5%; and antinuclear antibody ti-

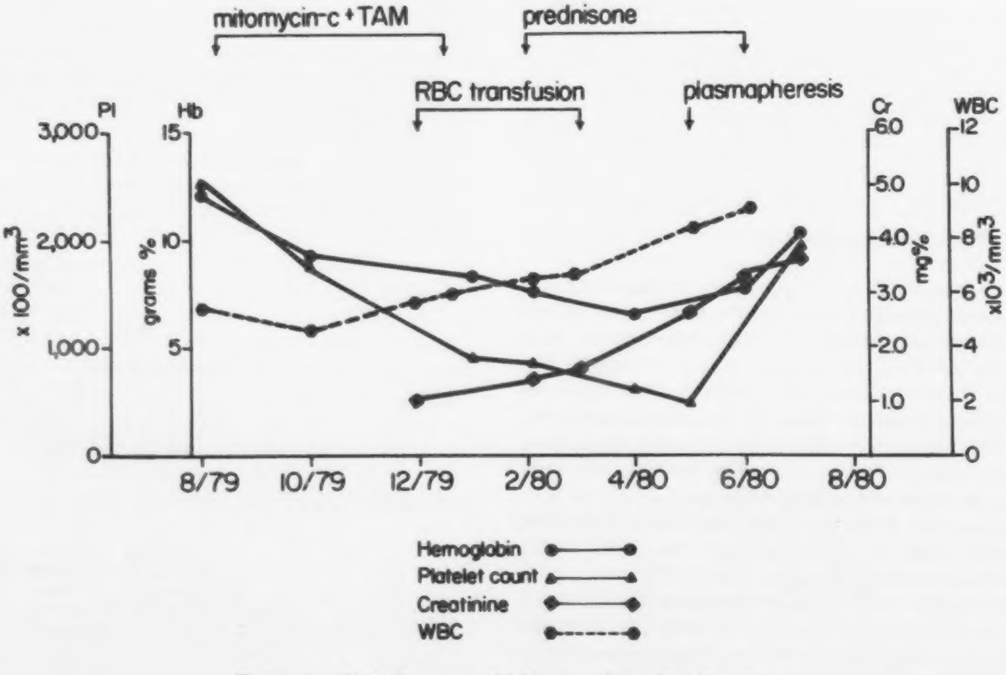

FIGURE 1.—Clinical course and laboratory data of study patient.

Table 1.—Comparison between previously reported cases and study patient*

	Case								
	1 (ref 2)	2 (ref 2)	3 (ref 3)	4 (ref 3)	5 (ref 4)	6†	7 (ref 5)	8 (ref 5)	9–14 (ref 6)‡
Primary tumor	Gastric CA	Gastric CA	Epidermoid CA of anal canal	Epidermoid CA, primary undetermined	Breast Ca	Breast CA	Undifferentiated CA	Lung adenocarcinoma	Adenocarcinoma
5-FU	+	+	+	+	–	–	–	–	
MM-C	+	+	+	+	+	+	+	+	
Vitamin B_{12}	+	+	–	–	–	+	–	–	–
TAM	–	–	–	–	–	+	–	–	–
Medroxyprogesterone	–	–	–	–	+	–	–	–	–
MAHA	+	+	+	+	+	+	+	+	+
Reticulocytosis	+	+	+	+	+	+	+	+	Unknown
Thrombocytopenia	+	+	+	+	+	+	+	+	+
Progressive renal failure	+	+	+	+	+	+	+	+	+
Congestive heart failure and pulmonary edema	+	+	+	+	+	+	–	Unknown	+
Coombs' tests	–	–	–	–	–	–	–	Unknown	Unknown
Compatible renal histology	+	+	Unknown	Unknown	+	+	+	Not done	Unknown
Gingival biopsy	Not done	Not done	–	Not done	Not done	–	Not done	Not done	Not done
Plasmapheresis	Not done	Not done	Improved MAHA	Improved MAHA	Not done	Improved MAHA	No improvement	Not done	Not done
Steroids	Not given	No improvement	Not given	No improvement	No improvement	No improvement	Not given	Not given	Not given

*CA = cancer.
†Patient in this study.
‡Patients received FAM (5-FU, doxorubicin, and MM-C).

ter, negative. Urinalysis showed 3+ occult blood, 3–5 rbcs/high-power field, trace proteinuria, and no casts. Evaluations for disseminated intravascular coagulation, vasculitis, and metastatic carcinoma were all negative. Needle aspiration and biopsy showed a hypocellular bone marrow. Erythropoiesis and granulopoiesis were decreased but with normal maturation. Megakaryocytes were normal and there was no evidence of tumor invasion.

Following one of the blood transfusions, the patient developed severe congestive heart failure and pulmonary edema that responded to diuretics, digitalis, and oxygen therapy. She continued to complain of muscle spasms. Aspirin and prednisone were administered but were ineffective. In May 1980, the platelet count was 50,000/mm^3. Cardiac catheterization and myocardial biopsy, performed after platelet transfusion, showed pulmonary hypertension. Microscopic examination of the myocardial biopsy specimen revealed some muscle fibers containing vacuoles measuring 3–6 μ in diameter. There was no increase in interstitial tissue. A platelet-associated immunoglobulin G (IgG) (performed at Scripps Clinic, La Jolla, CA) was elevated. Gingival biopsy did not reveal hyaline thrombi. The fibrin-plasminogen slide (Todd method) was not performed. Plasmapheresis was started in May 1980. The patient improved and platelet-associated IgG disappeared. Hgb increased to 10.7 g/dl without transfusion, and platelet count increased to 175,000/mm^3. Serum creatinine, however, increased to 3.7 mg/dl, and urinalysis showed heavy proteinuria and many wbc casts. By light microscopy, a renal biopsy showed glomeruli with generalized and segmental subendothelial swelling. This, together with focal and segmental thickening of the capillary basement membrane and mesangial matrix expansion, had led to the narrowing of the capillary lumina. There was focal perivascular fibrosis. Scattered foci of tubular atrophy associated with few dilated tubules that contain hyaline casts were noted. Interstitial edema and mild fibrosis associated with few lymphocytic infiltrates were seen. The arteries and arterioles showed moderate intimal thickening. By electron microscopy, the most striking ultrastructural changes were marked swelling and expansion of the lamina rara interna that led to marked narrowing of the capillary lumina. The mesangial matrix was focally expanded, and the mesangial cells contained numerous lipid droplets. The visceral epithelial cells showed focal villous transformations with minimal fusion of the foot processes. Immunofluorescent stain showed minimal granular fibrinogen deposits along the capillary walls. No IgG, IgA, or IgM deposits were seen. The patient continued to receive diuretics and digitalis and salt was restricted. In August 1980, she was brought to the emergency room, where she died. A post-mortem examination was not performed.

MM-C-induced microangiopathic anemia with renal dysfunction had been previously reported. Table 1 shows a comparison between the previously reported cases and our patient. The similarity is striking. MM-C was the only common drug given to all patients. MAHA was the initial presentation of all patients. Renal and cardiopulmonary dysfunction appeared to progress rapidly with the initiation of blood transfusions. The MAHA in our patient and in two previously reported patients improved after plasmapheresis, but there was no improvement in the renal function. There was no improvement with corticosteroids in our patient or in three previously reported cases. Aspirin and prednisone were given to our patient without benefit.

We think that, in our patient, MM-C was most likely responsible for the occurrence of the hemolytic uremic syndrome and possibly the cardiopulmonary insufficiency. The repeated blood transfusions may have added to the hemolytic process with its deleterious effects on the kidneys. We therefore suggest that, in patients receiving MM-C who develop MAHA, the drug should probably be discontinued, blood transfusions should be withheld unless there is a life-threatening situation, and plasmapheresis with exchange transfusions should be promptly started. This approach may reverse the MAHA and prevent a catastrophic and potentially irreversible renal failure. The effectiveness of this suggested approach, however, remains to be verified.

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Sami J. Rabadi
Janardan D. Khandekar*
Harry J. Miller[1]

Division of Oncology and Hematology
Department of Medicine
Evanston Hospital
Evanston, IL 60201

Northwestern University Medical School
Chicago, IL 60611

November 16, 1981

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[1] We thank David Hueter and Jacob Bitran for participation in the management of this patient, Dr. E. S. Kurtides for critical review, and Mrs. Mary Steele for careful typing of the manuscript.

**Reprint requests to:* Dr. J. D. Khandekar, Evanston Hospital, 2650 Ridge Ave, Evanston, IL 60201.

Vindesine Overdose

A 67-year-old male with lung cancer, diagnosed in February 1980, was seen at our unit for consideration of further treatment after seven monthly courses of vincristine, doxorubicin, and cyclophosphamide received elsewhere. A tissue diagnosis of small cell cancer was obtained by bronchoscopy, and progressive disease was shown on chest X-ray. Treatment with cisplatin (60 mg/m^2) every 4 weeks and vindesine (VDS) (3 mg/m^2) weekly was started. Over 3 months the patient received a total of 300 mg of cisplatin and 54 mg of VDS.

Both hematologic and neurologic toxicity had been very low, and a good partial remission was shown radiologically. Inadvertently, Dose 13 of VDS, administered on April 6, 1981, was *45 mg* instead of 4.5 mg as intended.

A few hours later, generalized muscle pain and tinnitus occurred, causing absolute sleeplessness for two nights. Sphincteric incontinence with diarrhea lasted for 48 hours, complete inability to swallow because of a burning sensation in the mouth and throat lasted 4 days, and hiccups resistant to medication lasted 16 days. On admission (April 9), body temperature was 37.2°C; pulse rate, 104 beats/minute; and blood pressure, 160/90 mm Hg. Blood chemistry was normal; wbc count, 3000/µl; platelet count, 55,000/µl; Hgb, 11 g/100 ml; reticulocytes, 0.1%; and serum iron, 220 µg/100 ml.

Oral nutrition was reinstituted on April 12; at this time tendon jerks had completely disappeared and fever began, accompanied by heavy drowsiness. On April 15, spontaneous micturition stopped; BUN had increased to 1.01 mg/100 ml, but in 24 hours it reverted to 0.29 mg/100 ml after positioning of a vesical catheter, which was needed for 10 days. Fifteen days after the overdose, serum iron abruptly decreased to 30 µg/100 ml, and reticulocytes increased to 3%; fever disappeared and the patient was again mentally alert. Gait and cranial nerve function were unimpaired.

ECG on Days 4 and 15 following overdose was normal. The wbc count nadir (1000/µl) and the platelet count nadir (50,000/µl) were attained on Day 10; wbc count reverted to 8500/µl and platelet count reverted to 150,000/µl by Day 16. No changes in serum Ca, Mg, K, Na, and Cl have been detected despite close surveillance. About Week 6 after overdose, ability to walk unaided was slowly regained, and tendon jerks were still absent. On Day 62 the patient had electromyography, which showed slowing of motor nerve impulses and partial denervation of muscles of the lower limbs. He was walking unaided and enjoying moderate outdoor activity by Day 82.

The supportive treatment, other than parenteral nutrition for 2 weeks, included 24 mg of leucovorin four times per day on Days 4–16 [on the basis of previously reported suggestions (1)], as well as 400 mg/day of citicolinum, 600 mg/day of citrulline, 1200 mg/day of arginine, and 600 mg/day of ornithine on Days 9–16. Improvement of consciousness and swallowing was noted 1 day before the patient's temperature decreased to normal and 1 day after the administration of citicolinum plus amino acids. In view of the previous leucovorin and of the concomitant disappearance of fever, it is difficult to assess whether these other compounds, known to interfere with nervous and muscular metabolism, contributed to the improvement in CNS and neuromuscular functions.

Bronchoscopy on Day 90 showed persistence of disease, which was treated by radiation therapy. At 6 months the appearance of supraclavicular adenopathy confirmed activity of disease, while the general condition was good, including mental status and muscular and sphincteric functions.

This case report shows that a VDS overdose of ten times the normal range is dangerous and requires close medical care but may be reversible. In table 1, the toxic effects, duration, and treatment in this VDS overdose are compared with those in a

TABLE 1.—Toxic effects, duration, and treatment in VDS overdose compared to vincristine overdose

Toxic effects in order of appearance	Onset	Duration (days)	Expected duration after vincristine overdose (days)*	Support treatment†
Muscular pain	Hour 4	3	7–28	Oral analgesics
Tinnitus	Hour 4	2	‡	
Impaired swallowing	Hour 6	4	‡	Parenteral nutrition with sugar, salts, and vitamins
Sphincteric incontinence	Hour 12	2	‡	
Hiccups	Hour 12	15	‡	Leucovorin
Hematologic toxicity	Day 3	12	7–21	Citicolinum + arginine + citrulline + ornithine (amino acids of NH_3 terminal disposal)
Inability to stand	Day 1	60		
Unconsciousness	Day 6	5	14–42	
Peripheralneuromyopathy	Day 7	70	3–30 +	
Fever	Day 7	5	1–3	Antibiotics
Anuria	Day 9	10	‡	Urinary catheter

*Ref 1.
†Support treatment used for our patient.
‡Toxic effects not previously described for vincristine.

vincristine overdose previously reported by Grush and Morgan (1). Compared with a similar episode following vincristine overdose (2), the blood cell count recovery occurred earlier in our patient, while the neurologic recovery took approximately the same time.

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2. BERENSON MP. Recovery after inadvertent massive overdose of vincristine (NSC-67574). Cancer Chemother Rep 55:525–526, 1971.

M. V. Fiorentino
L. Salvagno
V. Chiarion Sileni
A. Paccagnella
E. Ferrazzi
V. Zagonel
V. Fosser

Divisione di Oncologia Medica
Ospedale Civile Padova 35100, Italy

December 30, 1981

ANNOUNCEMENTS

Fellowships in Cancer Chemotherapy—Institut Jules Bordet

Brussels, Belgium

Applications are invited for two yearly fellowships in cancer chemotherapy in the Medical Department of Institut Jules Bordet, the cancer center of the University of Brussels. The Chief of the Department is Dr. Jean Klastersky. Candidates should be board-certified or eligible. The stipend is provided by the Foundation for Cancer Chemotherapy (FOCA).

For further information, contact: Dr. Henri Tagnon, President, FOCA Institut Jules Bordet, 125 Boulevard de Waterloo, 1000 Brussels, Belgium.

Approaches to the Management of Pain

Oakland, California, September 9, 1982

This one-day conference will deal with the multidisciplinary approach to the management of pain. Cosponsoring organizations are the Bay Area Tumor Institute and the East Bay Cancer Program. The program will be held in Goodman's Hall, 10 Jack London Square.

For further information, contact: Despina C. M. Johnson, MPH, Program Manager, 2844 Summit St, Suite 204, Oakland, CA 94609 (telephone: 415-465-8570).

Fourteenth Meeting of the International Society for Pediatric Oncology (SIOP)

Berne, Switzerland, September 21–25, 1982

The main theme of this meeting will be new modes of therapy and supportive care. Specific topics will include immune modulation, clinical applications of monoclonal antibodies, bone marrow transplantation, infection and nutrition, and new modes in surgery and radio-oncology. There will be reports on current trials as well as a pathologists meeting. The meeting, to be conducted in English, will be held at the Department of Pediatrics, Inselspital, 3010 Berne, Switzerland.

For further information, contact: Hans P. Wagner, MD, c/o SPOG, Institute for Clinical and Experimental Cancer Research, Tiefenauspital, 3004 Berne, Switzerland.

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices Although teaching will be primarily provided through lectures, panel discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Epidemiology—Methods and Applications

Chicago, Illinois, October 18–20, 1982

This course, sponsored by the New England Epidemiology Institute, will be held at the Ritz-Carlton Hotel in Chicago. Drs. Philip Cole and Kenneth Rothman will present modern concepts in epidemiology and their applications to the study of etiology, natural history of disease, and strategies in preventive medicine and public health. The course begins by providing a synthesis of analytic techniques that have been developed during recent years. The application of these techniques to research problems and disease prevention will be illustrated by examples from the medical literature. Students will learn the criteria by which epidemiologic studies of various types are assessed.

This course is intended for professionals in health and related disciplines who wish to develop a familiarity with epidemiologic research principles. This course is especially appropriate for persons not actively engaged in epidemiologic research but who are required to evaluate and interpret such research. No previous study of epidemiology or biostatistics is required. Registrants may receive Continuing Medical Education Credits (AMA Category 1) upon application.

For more information, contact: Dr. Nancy Dreyer, New England Epidemiology Institute, Dept SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Seventh International Symposium on Polynuclear Aromatic Hydrocarbons

Columbus, Ohio, October 26–28, 1982

This symposium, to be held at Battelle's Columbus Laboratories, has become known as important and highly relevant to the needs of workers in the area of polynuclear aromatic hydrocarbon research. It will feature invited lecturers, poster presentation, and simultaneous biology and chemistry sessions. Special sessions in the following areas are being organized: bioactivity of derivative species, carcinogenesis-mutagenesis, cell transformation, detoxification, inhalation of polynuclear aromatic hydrocarbon particulates, in vivo and in vitro metabolism, new biochemical techniques, and structure-activity.

For more information, contact: Dr. W. M. Cooke (telephone: 614-424-5024) or Dr. A. J. Dennis (telephone: 614-424-7068).

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NIH Publication No. 82-22

66/6

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cancer treatment reports

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June 1982
Volume 66
Number 6
Pages 1251-1453

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

HE 20. 3160:

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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

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COMMENTARY

Oral Complications of Cancer Chemotherapy: Present Status and Future Studies[1]

Douglas E. Peterson[2,*] and Stephen T. Sonis[3,4]

Chemotherapy has led to improvements in survival for leukemia, lymphoma, testicular cancer, small cell lung cancer, and other neoplasms. Myelosuppression is often associated with this treatment; the more profound and prolonged the myelosuppression, the more serious the complications that may develop. Management of these complications is critical for short-term treatment, patient comfort, and ultimately patient survival.

The oral cavity is a site in which complications secondary to cancer treatments frequently develop (1–7). However, current diagnosis and management of these sequelae within the oral cavity are relatively limited in scope. Variables such as disease type, treatment program orientation, health professional motivation, and recognition of the potential problems in part dictate the approach to these complications. Often the clinical situation in which oral pathology is not accurately diagnosed and treatment becomes inadequate leads to even more serious sequelae.

A recent symposium[5] representing medicine, dentistry, nursing, dental hygiene, and pharmacy identified several areas of chemotherapy-associated oral complications, both infectious (mucositis, periodontal infection, and dental pulp infection) as well as noninfectious (xerostomia, anemia, neurotoxicity, and thrombocytopenia). The purpose of this report is to review these sequelae, as well as avenues for their future research. This review should identify the need for the dental staff to play an integral role in the prevention, recognition, and treatment of oral sequelae. By incorporating such a staff as part of the overall oncologic team, the supportive care needs of the patient receiving chemotherapy will be better met.

INFECTIOUS COMPLICATIONS

Complications of the oral cavity are frequently seen following the administration of many cancer chemotherapies. Approximately 40% of all patients receiving drug therapy develop oral problems as a direct consequence of treatment (1). Oral complications may be caused by the nonspecific action of the chemotherapeutic agent on the rapidly dividing cells of the basal epithelium, resulting in ulceration and destruction of the mucosa (mucositis). Alternatively, chemotherapy-induced myelosuppression may exacerbate chronic oral infections such as periodontal or pulpal disease, produce local secondary infection of ulcerated mucosal tissue, or promote oral bleeding. Clearly, the loss of an intact epithelial barrier in a site which tends to be heavily colonized by potential bacterial pathogens may have serious consequences for the patient.

Mucositis (fig 1)

The development of mucositis in myelosuppressed patients is varied. Some chemotherapeutic agents are associated with a high incidence of oral mucositis (eg, methotrexate or doxorubicin), but not all patients receiving these agents develop even mild mucositis. The pathogenesis of mucositis is different from that of acute periodontal infection, which arises in a site of previous chronic infection; chemotherapy-induced oral mucositis arises on a usually intact mucosa. The drugs are directly toxic to cells which are undergoing rapid replacement (8–11); once the break in mucosal integrity occurs, secondary infection can readily occur. In addition, mucositis may

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[1] Received Feb 8, 1982; accepted Mar 29, 1982.

[2] Department of Oral Diagnosis, Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore; and University of Maryland Cancer Center, University of Maryland School of Medicine, Baltimore.

[3] Oral Medicine and Oral Pathology, Harvard School of Dental Medicine; Brigham and Women's Hospital; and Sidney Farber Cancer Institute, Boston, MA.

[4] We are grateful to Dr. Joseph M. Aisner for his editorial review, and we thank Ms. S. Henderson and Ms. S. Prignano for preparation of the manuscript.

[5] Oral Complications of Cancer Chemotherapy, Baltimore, MD, Sept 21–22, 1981.

*Reprint requests to: Douglas E. Peterson, DMD, PhD, Department of Oral Diagnosis, Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore, 666 W Baltimore St, Baltimore, MD 21201.

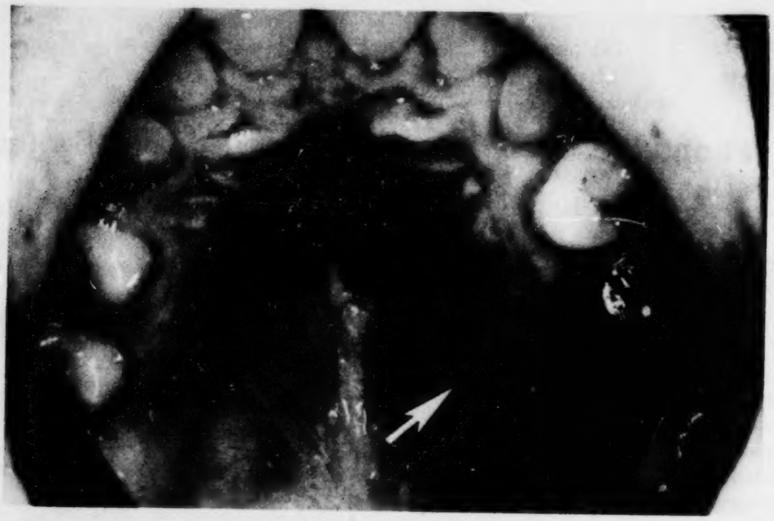

FIGURE 1.—Oral mucositis of the palate secondary to chemotherapy (arrow). This lesion appeared approximately 1 wk after initiation of therapy on tissues which were clinically normal prior to the treatment (photo courtesy of Ms. Lisa T. Williams, RDH).

impair the patient's nutritional intake because of the discomfort of mastication and the burning sensation of some mildly acidic foods. Microbial cultures and appropriate antibiotic therapy should be considered for all such lesions that are acutely infected.

The treatment of oral mucositis in general remains palliative and symptom-oriented. There appear to be few ongoing prospective studies to determine susceptibility to the development of such a lesion, or ideally, the means by which the lesion might be prevented from initially developing. Oral hypothermia techniques are being evaluated in some centers; questions as to the effect of such procedures on antitumor treatment as well as lack of delivery to the remainder of the gastrointestinal mucosa persist. Although some studies have suggested that good oral hygiene reduces oral mucositis (12–14), this has not been our experience. Successful management ideally should include elimination of the toxic effects on the mucosa and prevention of the mucosal ulceration wherever possible. This prevention might include measures directed against oral microbial flora, since it has been shown that germ-free animals have mucosal cell renewal rates that are one half those of conventional animals (15).

Periodontal Infection (fig 2)

Periodontal complications occur more frequently than is generally realized. Periodontal disease is extremely prevalent, affecting up to 98% of the population of this country (16). This prevalence is also found in patients scheduled to receive myelosuppressive chemotherapy (table 1). While recent reports have described the impact of oral care protocols on infection in myelosuppressed patients (12–14,17–19), many oncologists appear reluctant to institute such care for fear of causing bacteremias in their patients. One should note that transient bacteremias can occur during mastication (20), in the presence or absence of clinical gingival disease (21,22). The prevalence of periodontal infection, its management, and its possible prevention in myelosuppressed patients with acute nonlymphocytic leukemia (ANLL) have been documented (6,7). Approximately 28% of all acute infections which developed during remission induction represented acute exacerbations of pre-existent periodontal disease. In most cases, the inflammation associated with this lesion was markedly suppressed, similar to the lack of inflammation at other sites in this clinical setting (23,24). Thus, the diagnosis is more difficult to make, and one must have a high index of suspicion. Intensive oral hygiene before and during myelosuppressive therapy has proven efficacious in preventing many of these infections in patients with ANLL (6). This prophylactic therapy does not significantly increase other adverse clinical sequelae, such as esophageal, pharyngeal, or pulmonary infections. In patients with gingival infiltrate and engorgement

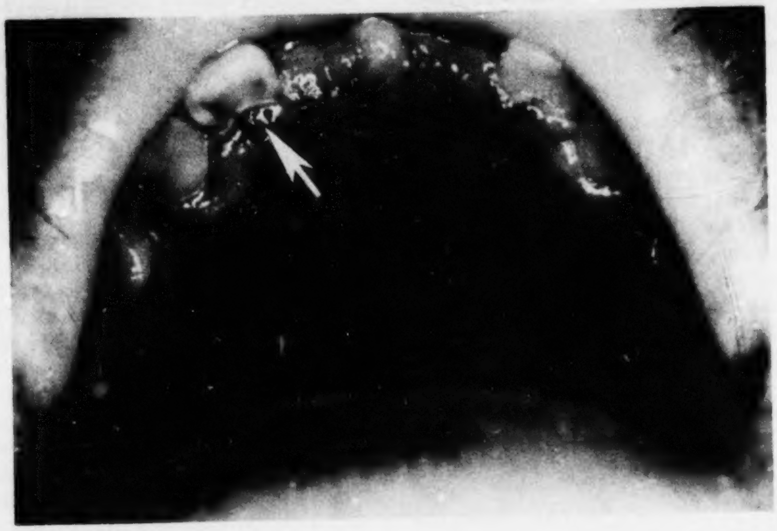

FIGURE 2.—Acute periodontal infection (arrow) in a granulocytopenic (< 100 cells/µl) patient with acute leukemia. Unlike oral mucositis, this lesion represents an acute exacerbation of a previously chronically infected site (photo courtesy of Ms. Lisa T. Williams, RDH).

secondary to acute monocytic or myelocytic leukemia or histiocytosis X, good oral hygiene can be extremely difficult to achieve. Such oral care does not appear to reduce acute periodontal infection in patients with leukemic infiltrates; further study as to effective oral care for this group of individuals is needed.

Since periodontal complications are infectious in nature, the clinician must be cognizant of changes in the oral microbial flora during treatment. Forty-seven percent of all acute infections in the myelosuppressed leukemic patient have been shown to be caused by organisms acquired in the hospital (25). It is also known that there is a shift in the oral microbial flora to enteric organisms as a consequence of disease and debilitation (26). Shifts similarly occur in the population of microbial flora within the oral cavity of patients with acute leukemia, with enteric gram-negative bacteria becoming predominant (27,28).

TABLE 1.—Status of periodontium at admission in patients with ANLL

Periodontal status	No. of patients
Healthy	0
Gingivitis only	2
Moderate disease	29
Severe disease	35
Total	66

It has been shown among immunodeficient patients that the supragingival plaque has higher levels of catalase-negative diphtheroids and *Candida sp.* than among normal individuals (29). There are, however, virtually no data available regarding the subgingival periodontal flora in patients receiving chemotherapy. It can be assumed that, similar to immunodeficient patients, there are probably profound changes in patients debilitated by or receiving myelosuppressive therapy. These changes are of major significance in treating both periodontal and mucosal infections in the myelosuppressed patient. Surveillance cultures are often used to monitor oral microbial changes in such patients (30). However, these techniques do not always directly sample flora contiguous to the ulcerative lesion of periodontal disease. Thus, a study (*a*) assessing shifts in the periodontal flora during myelosuppressive chemotherapy and (*b*) identifying etiologic agents causing acute periodontal infections has been instituted at the University of Maryland Cancer Center. To date, preliminary data from 20 patients suggest that the acute periodontal exacerbations are not caused by major pathogens associated with mortality in ANLL [eg, *Klebsiella sp.*, *Proteus sp.*, *Escherichia coli*, *Aspergillus sp.*, and *Pseudomonas sp.* (25,31)]. In addition, it appears that there is a quantitative but not qualitative shift in the microbial population during profound, prolonged granulocytopenia. Further study is needed to confirm and elaborate these find-

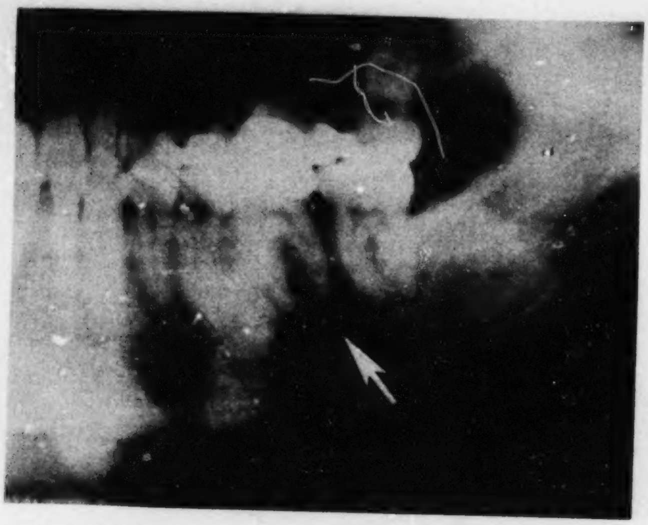

FIGURE 3.—Roentgenograph of periapical pathosis (arrow) of mandibular left first molar. This patient was granulocytopenic at admission, was febrile (38.3°C), and complained of intense pain in this region. This acute infection should be treated by either tooth extraction (if appropriate) or endodontic therapy (root canal).

ings. The techniques utilized in the analysis of such organisms are tedious and specialized; the periodontal flora are usually gram-negative anaerobes resident within the periodontal pocket (32,33) and are fastidious in their growth requirements. The laboratory must be equipped to culture these microbial agents; otherwise, accurate identification is not likely.

Dental Pulp Infections (fig 3)

Chronic infections of the dental pulp may also become a source of significant systemic infection during periods of myelosuppression. A thorough dental examination, including roentgenographs, is frequently the only way such pathology can be identified. Between 5% and 10% of cancer patients demonstrate roentgenographic evidence of pulpal infection of sufficient magnitude to cause bony involvement. The optimal treatment of involved teeth during granulocytopenia remains unresolved. However, elimination of the source of the infection, either by tooth extraction or by pulpal extrication, may often be the treatment of choice.

While these issues require further study, it is clear that tooth extractions in patients with profound, prolonged myelosuppression can be associated with serious systemic complications. However, a recent study has shown that by adhering to stringent guidelines the potential for such complications is minimized (34). Briefly, these guidelines include (*a*) antibiotic prophylaxis if the patient's granulocyte count is < 2000/µl, (*b*) use of effective platelet transfusions (ie, those which produce increments including matched platelets where needed) if platelet count is < 40,000/µl, (*c*) avoidance of intraalveolar hemostatic packing agents, (*d*) primary closure and alveolectomy at time of surgery, and (*e*) if possible, waiting at least 10 days between extraction date and induction of granulocytopenia (< 1000/µl) secondary to chemotherapy.

NONINFECTIOUS COMPLICATIONS

Noninfectious complications that can arise in patients receiving cancer treatment include xerostomia, anemia, neurotoxicity, and thrombocytopenia.

Xerostomia is a transient development secondary to some chemotherapeutic agents; the patient complains of a generalized dryness of the oral cavity. The lesion can be confirmed by milking of the parotid and submandibular glands and noting decreased quantity of salivary flow. In addition, the oral mucosal membranes will often appear atrophic and dry. The patient should be informed as to the temporary nature of the condition. Palliative relief can be obtained by the use of Xero-Lube (Scherer Laboratories, Dallas, TX), a glycerine-based solution containing sodium fluoride, or oral rinses with 0.1% aqueous sodium carboxymethylcellulose (35). Since a xerostomic condition may predispose to oral mucositis, it probably merits palliative topical therapy.

The most striking oral findings with anemia may be atrophic tongue and buccal mucosa, as well as generalized pallor. Obviously, this condition will persist until normal marrow function resumes. The direct effects of the chemotherapeutic agents may also predispose the patient to a neuropathy, with symptoms in the oral cavity that may mimic those of odontogenic or periodontal origin. In this clinical setting, the clinician should look for obvious oral disease, such as dental pulpal pathology or acute periodontal infection; if there is none, no active dental therapy is recommended, since the condition is usually reversible.

Thrombocytopenia per se rarely results in profound oral complications. Oral bleeding is a relatively common consequence and may occur in one of several forms. Gingival bleeding is seen with platelet counts $< 15{,}000/\mu l$ and is generally more severe in patients with pre-existing periodontal disease or poor oral hygiene. Hematoma formation as a result of trauma is often exaggerated when platelet counts decrease significantly below $50{,}000/\mu l$. Secondary infection of areas of hematoma are also a concern in the myelosuppressed patient. Finally, extravasation of blood into mucosal tissues, especially sublingually, may occur, but respiratory embarrassment is a rare sequela.

To control minor local oral bleeding, topical thrombin (100–100,000 units/ml) can be used. It should be remembered that, even in a thrombocytopenic patient, gingival bleeding can indicate inflammation. Failure to maintain excellent oral hygiene can result in increased oozing compared to that seen with the proper use of oral hygiene instruments, such as toothbrush and dental floss. Appropriate platelet transfusions (random donor platelets for most and matched platelets for immunized patients) can usually stop most of this type of bleeding.

FUTURE DIRECTIONS

The major goals of oral care in the cancer patient receiving chemotherapy are: assessment and treatment prior to chemotherapy, maintenance of good oral hygiene, patient education, and diagnosis and treatment of oral complications. Dental services should be part of the routine care of the patient with cancer. In many cases, the size and scope of the facility will dictate the extent to which this care can be provided. The dental staff should be contacted and those with interest and knowledge in the field should be recruited to evaluate and administer oral treatment to patients receiving chemotherapy. In institutions with teaching services, dental residents and attending staff should be afforded facilities necessary to provide comprehensive diagnosis and treatment of odontogenic pathology and management of soft tissue complications. If no hospital-based dental program exists, the patients could possibly be referred to their own dentists for evaluation; however, the oncologist should clearly specify to the dentist the nature of the concerns.

The future of supportive oral care for the patient receiving chemotherapy lies in a comprehensive cooperative team approach. To improve the ability of the dental team in providing basic supportive services, there are four areas that require further investigation.

1. Almost all clinical decisions regarding oral complications are currently based on limited data. There is a major need for prospective, well-controlled clinical studies on the subject. These studies should include analysis of (*a*) complications of dentulous and edentulous patients, (*b*) topical local therapy to reverse or prevent complications arising from systemic therapy, (*c*) new approaches to local control of infection and bleeding resulting from myelosuppression, (*d*) long-lasting topical palliative rinses, and (*e*) effect of oral hygiene on complications of chemotherapy.
2. There is a need for a classification system for oral complications of chemotherapy, since currently there are no standards for collection and analysis of such data. As a result, data cannot easily be compared among institutions. It is conceivable that a national registry for reporting, documentation, and analysis of oral complications of chemotherapy may be developed to allow data sharing.
3. Animal models need to be established to evaluate variables affecting the frequency and severity of oral complications as well as methods for therapeutic intervention.
4. Education of health care professionals concerning the need for a multimodality approach to the prevention and treatment of oral complications is essential.

The field of oral complications of cancer chemotherapy has had a comparatively limited development. Further research in this area will provide for better patient care.

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Chemotherapy Sensitivity Assessment of Leukemic Colony-Forming Cells With In Vitro Simultaneous Exposure to Multiple Drugs: Clinical Correlations in Acute Nonlymphocytic Leukemia[1,2]

Chan H. Park,[3,*] Mammo Amare,[3] Francis S. Morrison,[3] Thomas R. Maloney,[3] and J. Wendall Goodwin[3,4,5]

SUMMARY

For 13 patients with acute nonlymphocytic leukemia receiving four-drug combination chemotherapy (14 chemotherapy trials), leukemic bone marrow cells were obtained before treatment and were exposed in vitro to all four-drug-mixture solutions simultaneously followed by the survival assessment of leukemic colony-forming cells (L-CFU). Survival of normal marrow colony-forming cells (CFU-C) was also assessed concurrently, and the sensitivity index (SI) of leukemic cells was determined as the ratio of the survival of CFU-C to that of L-CFU. Correlations of in vitro results to clinical results were excellent: four of five trials resulting in complete remission had high SI and eight of nine trials without complete remission had low SI ($P = 0.02$). The potential advantages of this method of exposing cells to drug mixture are discussed in terms of detecting drug synergism and improving the efficiency of in vitro chemotherapy sensitivity studies.

[Cancer Treat Rep 66:1257–1261, 1982]

Our recent studies indicate that in vitro chemotherapy sensitivity of leukemic colony-forming cells (L-CFU) correlates well with clinical response to the same chemotherapy in individual patients with acute nonlymphocytic leukemia (ANLL) (1,2). All nine patients reported in our last study received combinations of two to four chemotherapeutic drugs, while in vitro chemotherapy sensitivity was assessed with component single drugs separately (2). Synergistic effects of component single drugs appear to be important in combination chemotherapy, and this synergistic effect is not reflected in vitro unless cells are exposed to the drugs together. The most straightforward initial step in this direction involves the use of mixture solutions of all the component single drugs. There are added advantages of using a drug mixture. Testing only one solution instead of multiple solutions of single drugs reduces the time, effort, expense, and number of cells required. Therefore, the logical extension of our last study with single drugs was to investigate multiple-drug sensitivity of L-CFU.

MATERIALS AND METHODS

Patients

Patients with newly diagnosed ANLL, without prior treatment, were candidates for this study. They

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[1] Received Aug 3, 1981; revised Jan 5, 1982; accepted Jan 28, 1982.

[2] Supported by Public Health Service grant CA-20717 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Chan H. Park is the recipient of Research Career Development Award CA-00534 from the Department of Health and Human Services.

[3] Department of Medicine, University of Kansas Medical Center, Kansas City (*C. H. Park* and *M. Amare*); Department of Medicine, University of Mississippi Medical Center, Jackson (*F. S. Morrison*); and Wilford Hall Medical Center, Lackland Air Force Base, TX (*T. R. Maloney* and *J. W. Goodwin*).

[4] *Present address:* Smith and Glynn-Calloway Clinic, Springfield, MO.

[5] We thank Drs. B. F. Kimler and K. Gerald for valuable advice and L. Payne, J. Bayer, and D. Menon for excellent technical assistance.

[*] *Reprint requests to:* Dr. Chan H. Park, Department of Medicine, Division of Clinical Oncology, University of Kansas Medical Center, 39th and Rainbow Blvd, Kansas City, KS 66103.

TABLE 1.—Clinical and in vitro chemosensitivity data of 13 leukemic patients

Patient/case No.	Age (yrs)	Sex	Diagnosis*	Treatment	Complete remission†	SI‡			
						Drug-mixture		Single drugs	
						SI-Mxa	SI-Mxb	SI-Cy	SI-An
1	69	M	AMML	AdOAP	Yes	2.91	3.49	0.97	3.0
2	51	F	AMML	ROAP	Yes	1.74	2.47	2.82	3.35
3	56	F	AMML	ROAP	Yes	1.89		1.5	1.2
4	45	F	AML	ROAP	Yes	5.92	6.73	0.64	0.88
5§	44	F	AML	AdOAP	Yes	0.69	0.87		
6	56	M	AMML	ROAP	No	0.57		1.03	0.96
7¶	59	M	AML	AdOAP	No	0.95		1.04	0.68
				ROAP	No	0.87		1.04	1.23
8	57	F	BT-CML	ROAP	No	0.95	0.91		
9	19	M	AMML	ROAP	No	0.61	0.74		
10	55	F	AML	ROAP	No	0.95	0.95		
11	69	F	AMML	AdOAP	No	0.79	0.81	0.72	0.63
12**	51	M	AML	ROAP	No	0.79	1.15	0.83	0.95
13††	62	M	AMML	ROAP	No	1.27	0.92	1.18	1.31

*AMML = acute myelomonocytic leukemia, AML = acute myelocytic leukemia, and BT-CML = blast transformation of chronic myelocytic leukemia.

†Complete disappearance of all evidence of disease, with normal marrow (blast cells < 5%).

‡In vitro chemotherapy sensitivity index (SI) calculated on the basis of either 1 concentration (SI-Mxa) or 4 concentrations (SI-Mxb) of drug mixtures and single drugs, cytarabine (SI-Cy) and anthracycline (SI-An) (*see* Materials and Methods).

§Good complete remission achieved with a long survival (23+ mos). However, the plating efficiency was low (12.4 ± 1.5 colonies/5 × 10^5 cells plated).

¶Patient failed therapy both with AdOAP and later with ROAP.

**Partial remission achieved with fairly good survival (13+ mos).

††Patient died in 3 mos with no evidence of response.

were given intensive combination chemotherapy, consisting of vincristine, cytarabine, and prednisone with either doxorubicin (AdOAP) (3) or zorubicin (ROAP) (2), to induce complete remission. Bone marrow was aspirated before chemotherapy for routine morphologic evaluation and in vitro chemotherapy study (see below). The subjects of this analysis are the initial 13 patients for whom both clinical response is evaluable and in vitro data are available (table 1). Complete remission in five patients from a sample of 13 patients is statistically acceptable assuming a true response rate of 60% for the population ($P = 0.10$ on the basis of binormal distribution). Normal bone marrow for control culture was obtained from hematologically normal patients with solid tumors undergoing bone marrow examination as part of the pretreatment staging workup. Informed consent was obtained prior to marrow aspiration from all patients in accordance with guidelines established by the University of Kansas Human Subjects Committee.

Cell Culture Assay

A detailed description of the cell culture method used in this study has been reported previously (4). Briefly, the culture system consisted of two layers of 0.3% agar in a 35-mm plastic Petri dish perforated at the bottom by five small holes. The top agar layer contained twice-washed buffy coat cells separated from bone marrow aspirates. Both layers contained Alpha medium (GIBCO, Grand Island, NY) with 15% fetal calf serum (Flow Laboratories, Rockville, MD) and 15% leukocyte-conditioned medium. The latter was prepared by incubation of normal human peripheral leukocytes with phytohemagglutinin (Wellcome Research Laboratories, Beckenham, England). Cultures were incubated for 2–3 weeks at 37°C in an atmosphere continuously flushed with 7% CO_2. Daily throughout the incubation period, each culture dish was removed from the incubator and fed from the top with 0.5 ml of Alpha medium containing 15% each of fetal calf serum and leukocyte-conditioned medium. The fed medium diffused through the agar layers and drained out of the dish through the holes on the bottom. Colonies of ≥ 50 cells were counted using an inverted microscope. Quadruplicate dishes were routinely set up for each experimental group of cultures, and the mean numbers of colonies were used to calculate chemotherapy sensitivity indices (see below). Plating efficiency varied considerably among different patients, and the cultures were set up initially with 5 × 10^5 cells (maximum number of cells which could reasonably be plated in a culture dish) to determine the plating efficiency for individual patients. If the number of colonies was less than ten at this cell concentration, chemotherapy studies were not performed in that patient; five of 18 patients (28%) could not be studied for this reason. On the other hand, in patients with high plating efficiency the number of plated cells was appropriately reduced to yield between 50 and 300 colonies. The number of colonies obtained for 13 patients in this study ranged between 12.4 and 266 (median = 78) for between 2×10^4 and 5×10^5 (median = 2×10^5) cells plated. Bone marrow cells were routinely stored in liquid nitrogen, and all the culture studies were done on these frozen cells as described previously (2,5). Previous studies (2,6,7) have substantiated that colonies growing in this culture system from leukemic bone marrow are leukemic in origin (L-CFU), while normal marrow gives rise to normal myeloid colonies (CFU-C).

In Vitro Chemotherapy Sensitivity Determinations

Vincristine, cytarabine, and doxorubicin were obtained in pure powder form without preservatives from respective manufacturers (Lilly, Indianapolis, IN; Upjohn, Kalamazoo, MI; and Adria, Columbus, OH). Clinical preparations were used for zorubicin and prednisolone (a hydroxylated compound active also in vitro) obtained from the National Cancer Institute, Bethesda, MD. A 1-hour pulse exposure of cells to drugs was done as in the previous study (2) except that a four-drug-mixture solution was used in addition to single-drug solutions. The four-drug solutions contained either doxorubicin or zorubicin in addition to vincristine, cytarabine, and prednisolone depending on whether the patient received either AdOAP or ROAP. The concentration of drugs that singly reduced the average survival of CFU-C to 75% (1,2) was used as the basis in making a standard drug-mixture solution. It was reasoned that this concentration of drug would be most effective in detecting differences in effect on survival of CFU-C and L-CFU, when L-CFU is more sensitive than CFU-C. If lower concentrations are used, lethal effects of L-CFU might not be sufficiently detected while greater concentrations of drug would tend to diminish differences in survival between CFU-C and L-CFU (2). For vincristine and prednisolone, one fifth of these indicated concentrations was arbitrarily used, because these two drugs probably play a minor role in the induction of complete remission, and the indicated concentrations appear too high in view of pharmacokinetic data (8).[6] Individual drug solutions for these four drugs were prepared and stored separately and were combined in equal volumes immediately prior to experiments to make drug-mixture solutions. Thus, the standard drug-mixture solution contained 5×10^{-8} M vincristine, 1×10^{-5} M cytarabine, 1×10^{-4} M prednisolone, and either 1×10^{-7} M doxorubicin or 1×10^{-7} zorubicin. Depending on their availability, bone marrow cells from each patient were tested either with this standard drug mixture only or also with five, ten, and 20 times this drug concentration. Whenever a leukemic bone marrow was tested for L-CFU survival, a normal bone marrow was also tested simultaneously and in identical fashion for CFU-C survival. The average survival rates of CFU-C with one-, five-, ten-, and 20-fold drug-mixture concentrations were 70%, 40%, 20%, and 3%, respectively, with an approximate coefficient of variation of 20%–30%. Because of this day-to-day variation, simultaneous CFU-C control is needed, as fully discussed and substantiated previously (2), to obtain stable data for chemotherapy sensitivity of L-CFU, which can be expressed in relation to that of CFU-C as follows:

$$\text{Sensitivity index (SI)} = \frac{\text{\% survival of CFU-C}}{\text{\% survival of L-CFU}}.^{7}$$

For SI calculated on the basis of one-time drug mixture only (SI-Mxa), simply the ratios of survival fractions of CFU-C and L-CFU were taken. If the data from four different drug-mixture concentrations were used, the areas under the survival curves were calculated in the manner described (9) for CFU-C and L-CFU and the ratios of these two areas were taken to yield SI-Mxb. SI for single drugs, either for cytarabine (SI-Cy) or anthracycline (SI-An), were based on one drug concentration (2). As can easily be seen from the formula, SI values > 1 indicate that L-CFU are more sensitive than CFU-C, and SI < 1 indicate that L-CFU are more resistant than CFU-C.

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[6] Townsend RJ (Medical Bioavailability Unit, Upjohn Co, Kalamazoo, MI). Personal communication regarding prednisone-prednisolone pharmacokinetic data, 1981.

[7] The survival fractions of CFU-C and L-CFU can fluctuate from one experiment to another. However, this fluctuation tends to occur in the same direction and by a comparable magnitude for a given set of experiments. Therefore, the ratio of these two fractions (ie, the SI) remains relatively constant. This was systematically studied and proven in the previous study (2). One of the examples in case 3 with zorubicin is shown as follows: SI 1.20 (49%/41%), SI 1.21 (80%/66%).

RESULTS

Table 1 shows in vitro chemotherapy sensitivity of L-CFU along with clinical data on 13 leukemic patients in this study. There were 14 chemotherapy trials, however, because one patient (No. 7) had two trials. For ten patients, enough cells were available to test all four concentrations of drug mixture, and both SI-Mxa and SI-Mxb were calculated. For the rest, only one concentration was tested for SI-Mxa. Therefore, SI-Mxa were available in all trials while SI-Mxb were not. SI for single drugs (SI-Cy and SI-An) were also shown for comparison where available. All SI for single drugs except for patients 4, 12, and 13 have been published previously (2).

Analyzing on the basis of SI-Mxa, four of five trials resulting in complete remission were associated with high indices with only one false low (case 5), and eight of nine trials not resulting in complete remission carried low indices with one false high (case 13). This correlation is statistically significant ($P = 0.02$ by Fisher's exact test), with an overall accuracy of 86% (12 of 14 trials). There was no significant difference between SI-Mxa and SI-Mxb ($P > 0.1$ by paired t test) in terms not only of whether the index was > 1 or < 1 but also of the absolute magnitude of the values. There were only two instances where obvious discrepancies were noted (cases 12 and 13). Because these two discrepancies were in opposite directions of each other, the overall clinical correlation would be the same, even if SI-Mxb were used where available. Data on three cases in which false correlations were noted either by SI-Mxa or SI-Mxb were examined more closely. Patient 5 had a plating efficiency that was quite low, the lowest among all, and therefore the SI based on this plating efficiency is likely to be subject to statistical variations. Patient 12 showed considerable clinical benefit from the chemotherapy, and therefore the high SI (SI-Mxb) might be closer to true sensitivity than the low SI (SI-Mxa). No ready explanation can be found for case 13. SI based on single drugs are generally in fair agreement with SI-Mx. Among five cases where SI-Mxa were > 1, both SI-Cy and SI-An were also > 1 in three cases, and the average value of SI-Cy and SI-An was clearly > 1 in one case (No. 1). Case 4 was exceptional, with exceedingly high SI-Mx in spite of low SI-Cy and SI-An. In five situations where SI-Mxa were < 1, both SI-Cy and SI-An were < 1 (two cases), and the average of these two values was < 1 (two cases) or very close to 1 (case 7, ROAP).

DISCUSSION

Chemotherapy sensitivity assessment on the basis of in vitro colony formation appears to be a reliable indicator for clinical response to the same chemotherapy in human malignant diseases as shown by us for ANLL (2) and by others for a variety of solid tumors (9,10). This can be valuable not only in the selection of the best possible chemotherapy drugs for individual patients but also in the screening of new compounds for further evaluation.

There are, however, a number of complicated factors to be considered. One is that most of the human malignancies, whether leukemias or solid tumors, are currently treated with combination chemotherapy, although most in vitro tests have been done with individual drugs. The synergistic effect between component single drugs appears to be important in combination chemotherapy, and this effect will not be reflected in vitro by exposing cells to single drugs separately. Our study here involves exposing cells in vitro to the mixture of multiple drugs, which may reflect the synergistic effect and thus be a better overall indicator for clinical outcome. The result of this study indeed shows an excellent clinical correlation, thus establishing the feasibility of in vitro multiple-drug exposure. It is, however, difficult to determine to what extent the drug-mixture exposure reveals synergistic effect of component single drugs. There are a limited number of cases where single-drug and drug-mixture exposures can be compared directly, and the results of these two exposures are generally in fair agreement with each other. However, there is a patient (No. 4) with good clinical response where SI-Mx is very high in spite of low SI-Cy and SI-An. This suggests the possibility of drug synergism detection by drug-mixture exposure. Also, in this approach where there is only one solution of multiple drugs to be tested rather than multiple solutions of single drugs, the number of cultures to be set up and the requirement of bone marrow cells are also considerably reduced. This reduces the expense of the study and can be an extremely important practical advantage for patients from whom enough cells are not obtained and/or for whom plating efficiency is low.

We intended to test four different concentrations of drug mixture in all patients. However, enough cells were not available in three patients, so only one concentration, the lowest, was tested. There was fairly good agreement between SI obtained on the basis of one (SI-Mxa) or four (SI-Mxb) concentrations of drug mixture. This also indicates that the values of SI are relatively stable with different drug concentrations. We would still like to test at least one more high concentration in addition to one low concentration, to detect the possible presence of minor cell subpopulations resistant to the drugs. However, if enough cells are not available, a test with one concentration appears to be acceptable.

The drug concentrations used in vitro with satisfactory results in our leukemia studies have been determined by a large number of experiments on CFU-C over many years (1). In more recent solid tumor studies of similar design by others (9,10), choice of drug concentration has been made on the basis of pharmacokinetic studies. It is interesting to note that the concentration of drugs used in both our leukemia system and in solid tumor studies (doxorubicin and vincristine) are remarkably similar. Further, the use of single rather than multiple concentrations of drugs also appears to be satisfactory in the solid tumor studies (10,11).

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Randomized Comparison of 5-FU Alone or Combined With Mitomycin and Cytarabine (MFC) in the Treatment of Advanced Gastric Cancer[1]

G. Cocconi,* V. DeLisi, and B. Di Blasio[2]

SUMMARY

In a prospective randomized study, 53 patients with advanced gastric cancer previously untreated by chemotherapy were randomly assigned to treatment with 5-FU alone or combined with mitomycin and cytarabine (MFC). The response rate with MFC was higher (28%) than with 5-FU alone (21%). The difference, however, was not statistically significant. Median response duration was practically identical with 5-FU (16 weeks) and MFC (17 weeks). There was no difference in median survival between the two groups (39 and 43 weeks, respectively).

[Cancer Treat Rep 66:1263–1266, 1982]

Combination chemotherapy is usually considered to be more effective in advanced gastric carcinoma than in any other gastrointestinal neoplasm. Combinations of 5-FU and nitrosoureas have been reported to produce objective response rates greater than 5-FU alone (1). Other subsequent phase II studies involving combinations of mitomycin and cytarabine (Ara-C) with 5-FU gave higher response rates than those usually seen with 5-FU alone (2). More recently, a 50% response rate has been reported in gastric carcinoma treated with the combination of doxorubicin, mitomycin, and 5-FU (3). Until now, however, there has been no evidence, based on a prospective randomized comparison, of a statistically significant superiority in the remission rate obtained by a combination of agents in comparison to 5-FU alone.

The purpose of the present prospective randomized study is to compare the efficacy of 5-FU alone to that of the combination of mitomycin, 5-FU, and Ara-C (MFC) in advanced gastric carcinoma.

MATERIALS AND METHODS

Patient Characteristics

Between January 1976 and December 1979, 53 consecutive patients with histologically proven gastric carcinoma were entered in this study. All patients had either advanced disease beyond the possibility of a surgical excision or a recurrence following primary surgical treatment. At least one measurable or evaluable parameter of disease, evidenced either directly or with instrumental examination, was required. Patients with an expected survival < 1 month, with other tumors, or with brain metastases were excluded. Laboratory requirements at the start of treatment were bilirubin < 3 mg/100 ml and creatinine < 1.5 mg/100 ml. Practically all patients were ambulatory. No patient had received previous cytotoxic chemotherapy or radiotherapy.

The 49 evaluable patients showed the following characteristics and distribution between the two treatment arms: median age was 64 years in both arms; 16 males and eight females received 5-FU and 19 males and six females received MFC; Karnofsky performance status was 100–80 in 16 and 17 patients, respectively, 70–50 in seven and eight, and < 50 in one who received 5-FU and in none who received MFC; the primary tumor had been resected in eight and nine patients, respectively, and unresected in 16 patients in each treatment arm; sites of indicator lesions were abdomen in six and five patients, respectively, liver in seven and ten, stomach in 16 and 17, and other sites in five and four. Treatment was assigned by randomization and was performed in our institution. Patients were directly and continuously followed by the same doctor during treatment and, if possible, during all subsequent chemotherapy regimens until death.

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[1] Received Feb 10, 1981; revised Nov 25, 1981; accepted Jan 5, 1982.

[2] Medical Oncology Service, Ospedali Riuniti, Parma, Italy.

**Reprint requests to:* Giorgio Cocconi, MD, Servizio di Oncologia, Ospedali Riuniti, 43100 Parma, Italy.

Treatment Procedure

Patients were randomized between two arms of treatment, 5-FU or MFC. They were stratified according to sex, age (< or > 60 years), and status of primary tumor. Drugs were given iv with the following schedules.

Arm A.—5-FU was given at the dosage of 13.5 mg/kg/day by bolus injection for 5 consecutive days, followed by a half-dose every other day until the appearance of toxicity. The treatment was then stopped and promptly resumed after recovery from gastrointestinal and hematologic toxicity. The treatment was then continued indefinitely, with 5-FU given at weekly doses of 15 mg/kg/iv.

Arm B.—MFC was administered twice a week for the first 2 weeks and then once a week indefinitely in the following doses: mitomycin, 0.08 mg/kg/iv; 5-FU, 10 mg/kg/iv; and Ara-C, 0.8 mg/kg/iv.

The dose of 5-FU was reduced if marked gastrointestinal toxicity appeared during the first 5 days of treatment. The following dose attenuation schedule for bone marrow depression was used in both arms: 100% of the dose for patients with wbc count $\geq 4000/mm^3$ and platelet count $\geq 120{,}000/mm^3$; 50% for wbc count between 3999 and $2500/mm^3$ and/or platelet count between 119,999 and $75{,}000/mm^3$; and temporary suspension of treatment for wbc count $< 2500/mm^3$ and/or platelet count $< 75{,}000/mm^3$. Hemoglobin measurements and wbc and platelet counts were performed before each dose, except on Days 2–5 of the first course of 5-FU.

Assessment of Response

At the time of entry in the study, a clinical examination was performed to measure and record any assessable tumor parameters. Abdominal masses and liver edge that were palpable under the costal margin were measured in centimeters and a map was drawn outlining the assessable tumor. At the same time, each patient had a routine biochemical examination, a liver scan and, if primary or recurrent tumor was present in the stomach, an upper gastrointestinal X-ray. Endoscopic examination was mandatory if tumor in the stomach was the only assessable parameter. Response to treatment was defined by the following criteria: *complete response (CR)*—the disappearance of all signs and symptoms of disease; and *partial response (PR)*—a ≥ 50% reduction in the sum of the product of the two largest perpendicular diameters of all measurable lesions. Liver metastases were used as indicator lesions only if proven by histologic or direct surgical assessment. Objective response was judged to have occurred when: (*a*) liver edge was initially measurable at least 5 cm from the costal margin and there was a decrease by at least 30% in the sum of measurements on the right and left midclavicular line and on the median line and/or (*b*) significant decrease in the diameter of liver lesions on the liver scan, from an initial diameter > 3 cm. Objective improvement (OI) was defined as tumor shrinkage > 25% but < 50%. In addition, it was required that all of the abovementioned therapeutic effects take place a minimum of 2 months from the beginning of treatment. Stable disease was defined as lack of growth of indicator lesions or volume changes < 25% for at least 2 months. Progressive disease (PD) was defined as a > 25% increase in any measurable lesion or the appearance of new areas of malignant disease.

Direct clinical examination was performed every month to assess therapeutic effects and to record toxicity. Liver and renal function tests were periodically repeated. Radiographic, endoscopic, and radioisotopic investigations were repeated every 2 months to assess clinical response. If the indicator lesion was primary or recurrent tumor in the stomach, evaluation of response required a repeated endoscopic examination, unless contrast radiography clearly showed PD. Duration of response was calculated from the beginning of treatment until objective proof of tumor regression was obtained. Survival was calculated from the beginning of treatment until death.

Statistical comparison of responses between the two treatments was performed by the chi-square method. Durations of response and survival were evaluated by the Kaplan and Meier method (4). Differences between distributions were tested using the Cox-Mantel test (5).

RESULTS

Toxic Effects

Analysis of nonhematologic toxic effects showed that they were more frequent in patients receiving 5-FU; nausea, vomiting, diarrhea, and stomatitis were observed in 58%, 41%, 29%, and 50% of the patients. These symptoms were generally mild to moderate and were generally observed after the initial 5-day course; occurrence was unusual during subsequent weekly administration of the drug. These gastrointestinal toxic effects were observed in a much lower percent of patients who received MFC (nausea and vomiting, 20%; diarrhea, 12%; and stomatitis, 8%). They were always mild and easily controllable with antiemetics. Alopecia was rare in all patients.

Analysis showed that hematologic toxicity was more frequent and severe with MFC than with 5-FU alone. The percents of observed episodes with 5-FU were lower than with MFC in reference to grade 1 (38% vs 70%) and grade 2 (0 vs 12%) leukopenia, and to grade 1 (25% vs 70%) and grade 2 (4% vs 40%) thrombocytopenia. Neither irreversible hematologic toxicity nor complications of myelosuppression (sepsis or bleeding) were observed. As a consequence of the more frequent hematologic toxicity with MFC, the percent of optimal dose administered to the patients was higher with 5-FU. The average percent of optimal 5-FU dose was high and stable during the first 6 months of treatment (98%, 100%, 100%, 97%, 97%, and 93%); with MFC, there was a tendency toward a progressive decrease (95%, 84%, 81%, 74%, 76%, and 69%).

Therapeutic Results

Four of the 53 patients entered in the study were considered to be nonevaluable for therapeutic response; one patient in each arm was lost to clinical controls before the first evaluation, and one patient in each arm died within the first 2 months, before the first assessment of response was performed.

A total of 24 patients who received 5-FU and 25 who received MFC were evaluable. In the 5-FU arm, 14 patients showed PD, three had no change, two had OI, four had PR, and one had CR. Thus, five of 24 patients treated with 5-FU showed an objective remission (21%). In the MFC arm, 13 patients showed PD, five had no change, none had OI, six had PR, and one had CR. Thus, seven of 25 patients treated with MFC showed an objective remission (28%). The difference in objective response rates between the two treatment arms was low and not statistically significant.

There was one CR in each treatment group. One patient treated with MFC achieved complete normalization of previous radiologic and endoscopic evidence of an inoperable tumor in the stomach, with multiple negative biopsies in the site where macroscopic and histologic evidence of tumor had previously been found. Second-look laparotomy was not performed. One patient treated with 5-FU had complete disappearance of metastatic lymph nodes in the left supraclavicular region.

Median duration of response was practically identical between 5-FU (16 weeks; range, 8–26) and MFC (17 weeks; range, 8–33). Median survival was slightly longer with MFC (43 weeks) than with 5-FU alone (39 weeks). The difference was not statistically significant. Responding patients (CR plus PR) had a longer median survival than patients with OI, no change, or PD (5-FU, 47 vs 39 weeks; MFC, 43 vs 22 weeks). These differences did not reach statistical significance, however.

Seven patients were crossed to MFC after PD or relapse on 5-FU. No objective responses were observed: there was OI in one patient, no change in two, and PD in two. Two patients were nonevaluable because of death before the first evaluation of response.

DISCUSSION

The combination MFC was first proposed by Hoshino et al (6) in L1210 mouse leukemia. In 1972, Ota et al (2) reported a 55% response rate in patients with stomach cancer. Even though the additive role of Ara-C to the activity of the combination is questionable or improbable because this drug is inactive in gastric carcinoma, a number of subsequent studies always utilized combinations including all three drugs to allow a comparative evaluation of results (7–9).

We observed a marked difference in type and degree of toxicity between the two treatments. 5-FU induced more frequent gastrointestinal disturbances, especially during the initial loading dose. With MFC, bone marrow depression was common and was manifested especially by thrombocytopenia, which often required a reduction in dose but rarely a transient suspension of treatment. There were no major complications such as fever or infections due to leukopenia, nor were there hematologic symptoms due to thrombocytopenia. Because there was little or no subjective impact induced by the moderate hematologic toxicity, MFC treatment was usually well-accepted by the patients.

Our data showed that MFC induces a response rate of the same order as that observed with 5-FU alone. There was no statistical difference, and it is impossible to speculate whether the slight increase in response rate observed with MFC compared to 5-FU should be attributed to the combination of mitomycin with 5-FU. Because the increase is so small, a statistical resolution of the problem is impracticable. However, the response rate (28%) obtained in this study with the MFC regimen is quite similar to that (31%) referred to in a study of patients with gastric carcinoma by De Jager et al (7) and higher than that (17%) reported by the Gastrointestinal Tumor Study Group (8). In any case, after the first optimistic report of Ota et al (2), all response rates observed with the MFC combination were at about the level usually expected with 5-FU alone. The present study confirms this observation by a direct prospective randomized comparison.

There was no evidence of any difference in duration of response between MFC and 5-FU. Median survival was higher with MFC than with 5-FU, but the difference was not statistically significant. The length of survival was rather high in both arms of this study and clearly longer than that reported for incurable untreated gastric cancer (10). This fact could be due to the rather high performance status of patients entering this study. It should be noted that patients treated with 5-FU were crossed to MFC when they had PD or relapse. With this sequential approach it was possible to achieve some positive results; OI was observed in one evaluable patient, and no change was observed in two among seven evaluable patients. The activity of MFC in patients pretreated with 5-FU was more clearly shown in the study of De Jager et al (7), in which three of 11 patients pretreated with 5-FU had PR with MFC.

Our data do not confirm the previously reported high response rate obtained by the MFC combination in advanced gastric cancer. It should be noted that our dose regimen of MFC was different from that used by the Gastrointestinal Tumor Study Group (300 mg/m^2/day of 5-FU, 1 mg/m^2/day of mitomycin, and 50 mg/m^2/day of Ara-C, all given on Days 1–5). Although that regimen (8) may be more intensive chemotherapy, the reported response rate was 17% (three responses among 18 patients). However, the MFC regimen we used, even if it is not statistically superior to 5-FU alone, may be one of the regimens useful for the treatment of gastric cancer, since a reasonable response rate with low subjective toxicity and relatively prolonged survival can be expected. MFC can also be useful in patients pretreated with 5-FU. Moreover, our study indicates that any good result obtained with combination chemotherapy in advanced gastric cancer should be controlled by a direct phase III comparison with 5-FU alone. In this regard, the role of MFC compared to the regimen containing 5-FU, doxorubicin, and mitomycin (FAM) deserves further clinical studies. These should take into account that the high response rates reported with FAM in the earlier studies (3,11) seem to be lower in subsequent evaluations or in other studies (12,13).

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Vincristine, BCNU, Doxorubicin, and Prednisone (VBAP) Combination in the Treatment of Relapsing or Resistant Multiple Myeloma: A Southwest Oncology Group Study[1,2]

J. Bonnet,[3] R. Alexanian,[4] S. Salmon,[5] R. Bottomley,[6] M. Amare,[7] A. Haut,[8] and D. Dixon[9,*]

SUMMARY

A total of 151 patients with myeloma who had relapsed with or were resistant to melphalan and/or cyclophosphamide (Cytoxan) with prednisone received vincristine, carmustine (BCNU), doxorubicin (Adriamycin), and prednisone (VBAP) at 21-day intervals; 39 patients (25%) responded. Of the 123 patients with relapsing myeloma, 37 (30%) responded. Only two (7%) of the 28 patients with resistant myeloma responded. The regimen was well-tolerated in patients who had not had prior extensive irradiation. Thrombocytopenia was the most frequent form of toxicity. Survival was significantly longer in those patients responding to VBAP (median, 78 weeks) than in those not responding (median, 33 weeks) ($P = 0.002$). VBAP is a safe, effective regimen for patients with myeloma who relapse with alkylating agents.

[Cancer Treat Rep 66:1267–1271, 1982]

Patients with multiple myeloma eventually fail to respond to melphalan and/or cyclophosphamide (Cytoxan) either singly or in combination. An alternative form of therapy is desirable for such patients.

A pilot study from the University of Arizona using carmustine (BCNU) and doxorubicin reported a response in seven of 13 patients in relapse after receiving a melphalan and prednisone combination (1). In a review of 462 patients treated with various combinations of alkylating agents, an increase in both response and survival was reported when vincristine was added to a combination drug regimen. Prednisone had previously been reported as having an additive effect on response rate.

Based on these observations a combination of vincristine, BCNU, doxorubicin (Adriamycin), and prednisone (VBAP) was used in patients with myeloma relapsing with or resistant to adequate trials of melphalan and/or cyclophosphamide with prednisone.

MATERIALS AND METHODS

This report presents an analysis of 151 previously treated patients with late-stage multiple myeloma seen at 28 Southwest Oncology Group (SWOG) institutions between December 1974 and August 1977. All 151 patients had progressive symptomatic myeloma. A previous response to melphalan and/or cyclophosphamide with prednisone had been documented in 123 patients. Response had consisted of a > 75% reduction in myeloma protein ("M") peak. All relapsing patients demonstrated progressive M peak elevations while receiving the therapy that produced previous response; 28 patients had shown

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[1] Received Mar 2, 1981; revised Dec 14, 1981; accepted Jan 21, 1982.

[2] Supported in part by Public Health Service grants CA-10187, CA-03195, CA-16957, CA-13612, CA-12644, CA-03392, CA-04919, CA-04915, CA-03096, CA-04920, CA-03389, CA-13238, CA-12213, CA-20319, CA-16385, CA-21116, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Scott and White Clinic, Temple, TX.

[4] University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[5] University of Arizona, Tucson.

[6] University of Oklahoma School of Medicine, Oklahoma City.

[7] University of Kansas Medical Center, Kansas City.

[8] University of Arkansas Medical Center, Little Rock.

[9] Southwest Oncology Group Statistical Office, Houston, TX.

* *Reprint requests to:* Southwest Oncology Group, Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

resistance to prior treatment with increasing M peaks.

Included in this analysis are 28 patients who had received < 90 mg/m² of either BCNU or doxorubicin sometime during the course of prior treatment with melphalan and/or cyclophosphamide.

Tumor mass prior to VBAP was rated as high, intermediate, or low, using criteria previously described (3).

Treatment Regimen

When eligible patients had recovered from the toxic effects of previous chemotherapy, VBAP was begun. Vincristine (1 mg) was administered iv, followed by BCNU and doxorubicin (30 mg/m² each). Prednisone (100 mg) was given orally for 4 consecutive days. Courses were repeated at 21-day intervals if there was no myelotoxicity. Treatment was delayed until the granulocyte count returned to 1500/mm³ and the platelet count returned to 100,000/mm³. For most patients, the delay was ≤ 1 week.

The doses of both BCNU and doxorubicin were decreased by 10 mg/m² if the granulocyte count nadir was < 1000/mm³, if the platelet count nadir was < 100,000/mm³, or in the face of an increasing BUN and/or creatinine.

Prior to each course, an ECG was performed and serum calcium, creatinine, uric acid, and BUN were measured. Anemia, hypercalcemia, and hyperuricemia were treated appropriately during the chemotherapy. An adequate trial was defined as three courses of therapy.

Response Evaluation

Clinical response was based principally on the decrease in the M peak or the myeloma protein production rate. Complete response (CR) was defined as the complete disappearance of the myeloma protein. Partial response (PR) was defined as a decrease of 75%–90% in the serum and/or urine M peak. The patients were rated as improved if there was a 50%–75% decrease in serum myeloma protein. No patient was rated as responsive if there was progression of other clinical indicators such as bone lesions, hypercalcemia, or azotemia.

Comparisons of response rates between subgroups were made using the chi-square test, survival curves were estimated using the method of Kaplan and Meier (4), and comparisons of survival duration curves were made using Gehan's generalization of the Wilcoxon test (5).

RESULTS

Five patients (3%) had CR, 20 (13%) had PR, and 14 (9%) were improved, for an overall response rate of 25% (39 responses among 151 patients). Twenty patients (13%) showed no change in the M peak, while 92 (62%) showed evidence of increasing disease. The response rate in relapsing patients was 30% (37 responses among 123 patients). There were only two responders (7%) among the 28 patients who had shown no response to previous therapy.

In the 39 responding patients a detectable decrease in the M peak did not occur until the patient had received at least three courses of VBAP. Thus, it was determined that three courses was the minimum number for adequate trial; 122 patients received at least three courses. The response rate in this adequately treated group was 32% (39 responses among 122 patients).

Of the 29 patients who did not receive an adequate trial of VBAP, 18 died, three refused further therapy, seven did not complete the trial due to toxic effects, and one was lost to followup before three courses were given.

The small amounts of BCNU or doxorubicin that 20 relapsing patients had received prior to VBAP did not significantly influence the response rate (table 1). However, the degree of response was notably

TABLE 1.—VBAP in relapsing or resistant myeloma: response*

	No. of patients treated	No. of responders (%)
Previous responders (relapse)	123	37 (30)
M–C	103	32 (31)
M–C + B or A	20	5 (25)
Previous nonresponders (resistant)	28	2 (7)
M–C	20	2 (10)
M–C + B or A	8	0 (0)
All patients	151	39 (25)

*M = melphalan, C = cyclophosphamide, B = BCNU, and A = doxorubicin.

TABLE 2.—VBAP in relapsing or resistant myeloma: response by type of M peak*

Protein	No. of patients	No. responding (%)	Response				
			CR	PR	IMP	NR	IND
IgG	65	17 (26)	2	6	9	12	30
IgA	37	5 (14)	0	2	3	5	27
BJ	32	12 (38)	1	11	0	2	23
IgG + BJ	15	4 (27)	1	1	2	1	11
IgD	1	0 (0)	0	0	0	0	1
Ig unknown	1	1 (100)	1	0	0	0	0
Total	151	39 (25)	5	20	14	20	92

*IMP = improvement, NR = no response, IND = increasing disease, and BJ = Bence Jones proteinuria.

TABLE 3.—SWOG myelotoxicity criteria

Grade	Nadir	
	Granulocyte count (cells/mm^3)	Platelet count (cells/mm^3)
1	2000–2499	75,000–99,999
2	1000–1999	50,000–74,999
3	500–999	25,000–49,999
4	< 500	< 25,000

different between the two groups. None of the patients who had relapsed after receiving prior BCNU or doxorubicin had CR, 10% had PR, and 5% were improved. Relapsing patients not previously treated with BCNU or doxorubicin had a 5% CR rate and a 17% PR rate, and 9% improved.

None of the patients in this study had low tumor mass. Ten (32%) of 31 patients with intermediate mass responded, and 29 (24%) of 120 patients with high tumor mass responded.

Table 2 shows response correlated with myeloma protein type. There was a significant difference in response rates in patients with IgA serum peaks and Bence Jones proteinuria. There was no significant difference in response rates among any of the other groups.

Toxicity

Myelotoxicity was recorded in 60 (40%) of the 151 patients (*see* tables 3 and 4). Thrombocytopenia was found in 46 patients (30%) but was reported to be a severe clinical problem in only ten (7%). There was one drug-related death. Nine of the 11 patients who had severe myelotoxicity had received radiotherapy to more than three sites prior to VBAP.

One patient had severe nausea and vomiting after the first course of VBAP and refused further therapy. Gastrointestinal toxicity of a significant degree was quite rare.

Two patients with increasing disease had a > 50% decrease in creatinine clearance after three courses of VBAP. It was not clear if this was due to drug toxicity or a complication of advancing disease. Both patients had light-chain disease.

One patient developed atrial fibrillation, and one showed marked ST-segment changes on ECG after the third course of VBAP. Both patients had advancing disease and received no further therapy; they had received only 90 mg/m^2 of doxorubicin.

Survival

Percentiles of survival from start of VBAP therapy were determined in 150 of the 151 patients in

TABLE 4.—VBAP in 151 patients with relapsing or resistant myeloma: myelotoxicity

Myelotoxicity	Grade				Fatal
	1	2	3	4	
Granulocytopenia	7	6	0	1	0
Thrombocytopenia	23	10	7	1	1
Pancytopenia	2	1	0	1	0
Total	32	17	7	3	1

this study. The median overall survival was 33 weeks, with 111 patients having died at the time of this analysis.

Objective response to VBAP was strongly related to survival. Of the 122 patients who had received enough treatment to have had a chance at response, the 39 responding patients had a median survival of 78 weeks compared with only 33 weeks for patients with no response. The survival curves for these two groups, as shown in figure 1, were significantly different ($P = 0.002$).

The original date of diagnosis was available; therefore, it was possible to calculate percentiles of survival from that date. The overall median survival was 166 weeks. Patients having received no previous BCNU or doxorubicin had significantly longer survival than those who had. Median survival times were 186 and 125 weeks, respectively ($P = 0.02$).

DISCUSSION

In previous SWOG studies (2), about 60% of the patients with untreated multiple myeloma achieved remissions as defined by a $\geq 75\%$ reduction in M peak or myeloma protein production rate. Using these same criteria, 16% of relapsing or resistant patients (25 of 151) responded to VBAP. An additional 14 patients (9%) showed a $> 50\%$ reduction in M peak and are included as responders in this evaluation. All 14 patients had been rated as responders in previous SWOG protocols and subsequently had shown at least a doubling of the M peak while receiving therapy, making them eligible for VBAP. As a result of VBAP, all 14 patients had an M peak or production rate reduction to the same level or below that at which they had been previously rated as responsive.

The response rate of 30% in patients with myeloma who had responded previously but were currently refractory was encouraging and demonstrates the utility of VBAP for some patients with progressive myeloma. Alexanian et al (6) reported that 80% of previously responding patients followed without chemotherapy until relapse responded to the resumption of melphalan and prednisone. Eventually, this group of responders failed to respond to continued melphalan and prednisone. In this relapsing group, Presant and Klahr (7) reported a 36% response (five responses among 14 patients) to a combination of doxorubicin (Adriamycin), BCNU, cyclophosphamide, and prednisone (ABC-P), using response criteria similar to those used in this study.

Adequately treated patients responding to VBAP showed a prolongation of median survival of 10 months over adequately treated nonresponders. Using ABC-P, Presant and Klahr (7) reported an increase in median survival of 6 months in responders versus nonresponders.

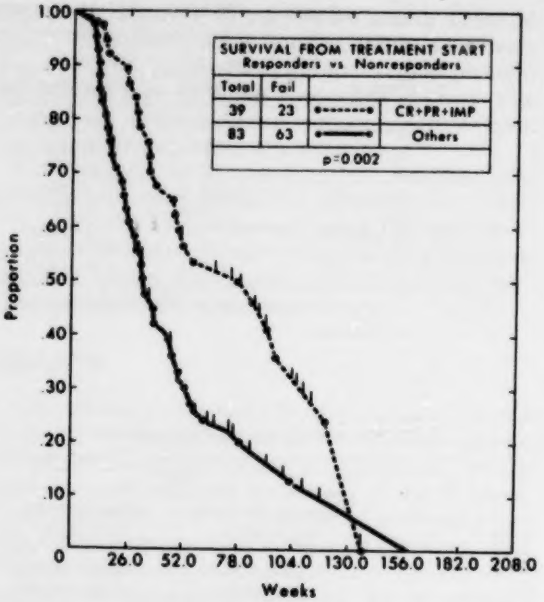

FIGURE 1.—SWOG-7432: survival in patients with relapsing or resistant myeloma treated with VBAP.

This study also confirms the efficacy of BCNU and doxorubicin combinations as reported in the pilot study reported by Alberts et al (1). While that study was small, a higher response rate was reported. The investigators did not employ a dose reduction of myelosuppressive drugs; patients were supported with granulocyte and platelet transfusions. The current study employed dose reductions. Therefore, there could have been less intensive remission induction in this study.

Using cyclophosphamide, BCNU, and prednisone, Kyle et al (8) reported a response rate of 17% (seven of 42 patients) in patients with myeloma resistant to melphalan. In that study, 7% (three of 47 patients) of the patients with myeloma resistant to melphalan responded to cyclophosphamide and prednisone without BCNU. The objective responders had significantly longer survival than nonresponders (31.0 vs 9.4 months; $P < 0.02$). Whether the apparent increase in response to BCNU and doxorubicin is due to the addition of doxorubicin to BCNU or to other factors such as dose scheduling or patient population cannot be determined from the available data.

The response of two (7%) of 28 patients with myeloma initially resistant to melphalan and cyclophosphamide is not impressive. In the pilot study, initial nonresponders to melphalan were also observed to be resistant to BCNU and doxorubicin (1). Since both studies have reported sensitivity or resistance to BCNU and doxorubicin (with or without vincristine and prednisone) in similar patient categories, it appears that a yet undescribed kinetic or biochemical factor distinguishes responders from nonresponders. Neither doxorubicin nor bleomycin alone has produced promising results in resistant myeloma (9). Although response to VBAP was impressive in one of the previously nonresponding patients, some other treatment regimen for these patients must be sought.

Prior exposure to BCNU or doxorubicin did reduce the rates of CR and PR to VBAP. BCNU or doxorubicin had been given prior to VBAP as part of maintenance protocols. This suggests the acquisition of resistance to these drugs at a time when the patient was considered to be in remission.

VBAP was tolerated in most patients, even those with late-stage disease. There was one drug-related death. Prior chemotherapy did not correlate with toxicity. There was some indication of a correlation between severity of disease and toxicity. The most significant finding was the correlation of severe toxicity to the previous amount of radiation therapy. In patients who had received radiation therapy to fewer than three sites, VBAP was well-tolerated.

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Phase III Study of Intermittent Carmustine (BCNU), Cyclophosphamide, and Prednisone Versus Intermittent Melphalan and Prednisone in Myeloma[1,2]

Neil Abramson,* Philip Lurie, William L. Mietlowski, Albert Schilling, John M. Bennett, and John Horton[2]

SUMMARY

A prospective randomized trial in patients with previously untreated multiple myeloma was performed comparing carmustine (BCNU), cyclophosphamide, and prednisone (BCP) to melphalan (Alkeran) and prednisone (AP). Induction response rates, remission duration, and survival were similar with both regimens. Hematologic toxicity was greater with AP. Crossover studies in patients who relapsed did not illustrate any significant activity with either drug treatment program. Therefore, BCP can be utilized as initial therapy in myeloma because of comparable remission rates and less hematologic toxicity.

[Cancer Treat Rep 66:1273–1277, 1982]

The intermittent melphalan (Alkeran) and prednisone (AP) regimen has been commonly used as induction therapy for multiple myeloma. Response rates of approximately 50% have been substantiated by many investigators (1,2) and the drug regimen is well-tolerated. Despite these results, it is necessary to improve survival by finding better induction and maintenance therapy. Encouraging preliminary results with carmustine (BCNU) and cyclophosphamide (3,4) prompted the Eastern Cooperative Oncology Group (ECOG) in 1972 to study (EST 4472) the combination of BCNU, cyclophosphamide, and prednisone (BCP) versus AP. Treatment groups were evaluated in terms of response rates, toxic reactions, crossover response rates, and survival.

METHODS

Untreated patients with multiple myeloma as judged by the guidelines of the Chronic Leukemia-Myeloma Task Force (5) were eligible if measurable

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[1] Received July 1, 1981; revised Feb 1, 1982; accepted Feb 11, 1982.

[2] This study was conducted by the Eastern Cooperative Oncology Group (CA-21115; *Paul P. Carbone*, Chairman) and supported by Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated: University of Florida-Jacksonville (CA-19818; *N. Abramson*); Sidney Farber Cancer Institute, Boston, MA (CA-23318; *P. Lurie* and *W. L. Mietlowski*); Boston University Hospital, MA (CA-11109; *A. Schilling*); University of Rochester Cancer Center, NY (CA-11083; *J. M. Bennett*); Albany Medical College, NY (CA-06594; *J. Horton*); Albert Einstein College of Medicine, Bronx, NY (CA-14958); University of Alberta, Edmonton, Canada; American Oncologic Hospital, Philadelphia, PA (CA-18281); Brookdale Hospital Center, Brooklyn, NY; Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Georgetown University School of Medicine, Washington, DC (CA-02824); Hahnemann Medical College, Philadelphia, PA (CA-13611); University of Iowa, Iowa City; Jackson Memorial Hospital, Miami, FL; University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, NY (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); University of Ottawa, Ontario, Canada; Centre Hospitalier de Bobigny, Paris, France (CA-19273); Pennsylvania Hospital, Philadelphia (CA-13613); University of Pittsburgh, PA (CA-18653); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); State University of New York-Stony Brook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Medical College of Virginia, Richmond (CA-10572); Yale School of Medicine, New Haven, CT (CA-15485).

**Reprint requests to:* Neil Abramson, MD, 820 Prudential Dr, Suite 511, Jacksonville, FL 32207.

disease was present (a monoclonal protein or plasmacytoma). Patients were stratified into good-risk (hemoglobin ≥ 9 g/100 ml, BUN ≤ 30 mg/100 ml, and calcium ≤ 12 mg/100 ml) and poor-risk (failure to meet good-risk criteria or expected survival < 6 months) categories. They were randomized to receive either (*a*) 75 mg/m^2 of BCNU iv over 30 minutes on Day 1, 400 mg/m^2 of cyclophosphamide by iv push on Day 1, and 75 mg/day of prednisone orally on Days 1–7 in a cycle that was repeated every 4 weeks or (*b*) 8 mg/m^2/day of melphalan orally on Days 1–4 and 75 mg/day of prednisone orally on Days 1–7 every 4 weeks. After six courses of therapy, patients were considered to have a partial response (PR) if serum monoclonal protein or plasmacytoma was reduced by 50% or urine monoclonal protein was reduced by 90% (in the presence of stable or improved renal function). Complete response (CR) was defined as the complete disappearance of measurable disease. Responding patients continued to receive the same induction regimen for 2 years. Patients not achieving a response or later failing initial therapy were crossed over to the alternate induction regimen. Survival data include all patients regardless of cause of death. Toxicity was judged according to standard ECOG criteria. Hematologic toxicity was considered severe when wbc counts decreased to < 2000/mm^3, granulocyte counts decreased to < 1000/mm^3, or platelet counts decreased to < 50,000/mm^3.

Tests for differences in discrete or frequency data were done with Fisher's exact test and Zelen's k-2×2 table methodology (6,7). Such data were also evaluated using the linear logistic model and multiple regression analysis (6). The "leaps and bounds" algorithm of Furnival and Wilson (8) was used to select the final regression model, with Mallows' Cp statistics as the selection criteria (9). Survival curves were drawn using the Kaplan-Meier method (10). Medians and survival percentages were determined from the survival curves.

RESULTS

Of 234 patients entered in the study, 188 were analyzed; 142 were evaluable and 46 were unevaluable, mostly because they lacked measurable disease or refused therapy. The group of unevaluable patients differed from the study group only in that it included fewer patients with hypercalcemia (35% as compared to 62%) and more blacks (36% as compared to 16%). Unevaluable patients did not differ in terms of other prognostic factors and had no statistically significant difference in survival. As shown in table 1, patient characteristics, except for sex, were equally distributed between the two treat-

TABLE 1.—Distribution of cases analyzed by on-study characteristics

		No. of patients (%)		
Category	Level	BCP	AP	Total
Initial performance status	0	22 (24)	15 (19)	37 (22)
	1	32 (36)	29 (36)	61 (36)
	2	11 (12)	13 (16)	24 (14)
	3	19 (21)	19 (23)	38 (22)
	4	6 (7)	3 (6)	9 (6)
	Not reported	6	11	17
Age	< 60 yrs	29 (31)	31 (34)	60 (32)
	60–70 yrs	37 (39)	33 (37)	70 (38)
	≥ 70 yrs	29 (31)	26 (29)	55 (30)
	Not reported	1	2	3
	Median age	64 yrs	63 yrs	64 yrs
Sex	Male	70 (73)	54 (59)	124 (66)
	Female	26 (27)	38 (41)	64 (34)
Race	White	79 (84)	76 (84)	155 (84)
	Black	15 (16)	14 (16)	29 (16)
	Not reported	2	2	4
Risk status	Good	62 (65)	54 (59)	116 (62)
	Poor	34 (35)	37 (41)	71 (38)
	Not reported	0	1	1
Prior surgery	No	85 (89)	84 (91)	169 (90)
	Yes	11 (11)	8 (9)	19 (10)
Prior radiotherapy	No	83 (86)	74 (80)	157 (84)
	Yes	13 (14)	18 (20)	31 (16)

TABLE 2.—Response rates for all evaluable patients by risk status

	No. of patients	
	BCP	AP
All patients		
CR	1	4
PR	34	27
No response	35	41
Response rate*	35/70 (50%)	31/72 (43%)
Good risk		
CR	0	3
PR	24	17
No response	24	23
Response rate*	24/48 (50%)	20/43 (47%)
Poor risk		
CR	1	1
PR	10	10
No response	11	18
Response rate*	11/22 (50%)	11/29 (38%)

*No. of CR + PR/No. of evaluable patients.

ment groups. Table 2 lists the response rates for the two therapies. For all patients, there was no significant difference in the response rates between BCP and AP. BCP treatment among 70 evaluable patients resulted in 35 CR plus PR, or a response rate of 50%, which was not statistically different from the 31 CR plus PR (43%) among 72 evaluable patients given AP treatment. There was one CR with BCP and four with AP. Response rates for good-risk and poor-risk patients demonstrated no significant differences between the two treatments. Performance status, age, BUN, calcium, sex, and race did not influence the response rates between the two treatment arms.

The toxicity of each treatment arm was categorized as severe or none to moderate. Table 3 shows the hematologic toxicity of BCP and AP according to pretreatment risk status. There were 21 patients excluded from this analysis because of lack of toxicity data. Although poor-risk patients had a higher percent of hematologic toxicity with AP as compared to good-risk patients, there was no statistical evidence of treatment-risk interaction ($P = 0.44$). However, when the group was analyzed as a whole, a statistically significant higher rate of severe hematologic toxicity occurred with AP than with BCP ($P = 0.03$). Gastrointestinal toxicity as judged by nausea, vomiting, or diarrhea was not greater in either treatment arm.

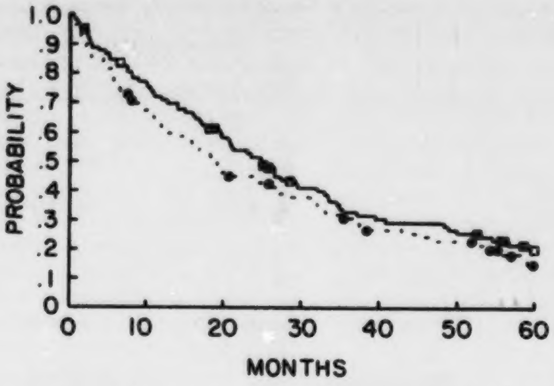
Survival was determined from the date of entry in the study and is shown in figure 1. Of 188 patients, 82% have now died. There was no difference in survival between the BCP- and AP-treated patients; median survival for the entire group was 21 months. Not illustrated is an improved median survival of 28 months for ambulatory patients as compared to 12.5 months for nonambulatory patients ($P = 0.01$), as well as improved survival in the group with better performance status and lower BUN. Initial calcium levels did not influence survival. There was no difference in survival between patients in the two treatment arms when separately analyzed by age, risk status, calcium, or BUN. The duration of response was evaluated from the onset of response and is shown in figure 2. The median length of response was 18 months for BCP and 24 months for AP; this difference was not statistically significant ($P = 0.88$).

Crossover data were evaluable in 47 patients. Only three of 28 patients receiving BCP in relapse responded as did three of 19 patients receiving AP in relapse; this difference was not significant.

DISCUSSION

The comparison of the BCP combination to the AP combination naturally followed the encouraging preliminary results with BCNU and cyclophosphamide used as single agents (4). In addition, 25%–30% of the patients who failed after melphalan treatment were reported to respond to yet another alkylating agent, cyclophosphamide (3). Furthermore, the combination of more than one alkylating drug given concurrently or sequentially was being studied in several cooperative groups (11–13).

In the study reported here, previously untreated patients with myeloma and measurable disease were randomized to receive BCP or AP. There was no difference in response rates between the BCP and AP groups. Furthermore, the duration of response

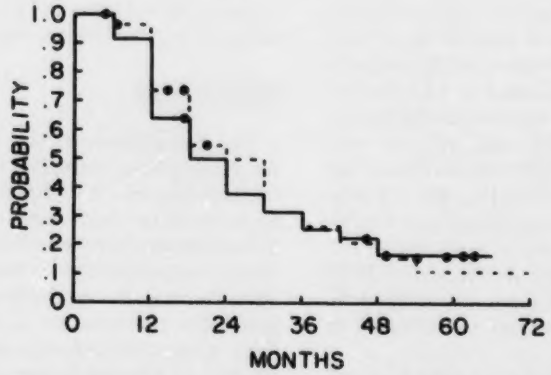


TREATMENT	ALIVE	DEAD	TOTAL	MEDIAN
—— BCP	19	77	96	24.6
----- AP	14	77	91	18.8

FIGURE 1.—Survival of patients according to induction treatment arm.

for the two groups was the same, which suggests that no greater tumor destruction was afforded by either therapy. In addition, survival of responding and nonresponding patients was unaffected by initial treatment choice. The response rate for the BCP arm in this trial is comparable to that initially reported by Cohen et al (11) for the same regimen. The use of AP in this trial resulted in more hematologic toxicity; however, as already shown, toxicity did not adversely affect duration of response or survival.

BCP can be considered as an acceptable alternative to the commonly used AP combination in the initial treatment of myeloma. In addition, the iv route is a more reliable method of delivering chemotherapy as compared to the oral route. Since BCP has less hematologic toxicity, one could consider adding other induction drugs to this regimen in future trials. Crossover data showed few secondary responses with either regimen, which suggests lack of cross-reactivity among alkylating agents. However, patients receiving crossover drugs in this study



TREATMENT	CONT	FAIL	TOTAL	MEDIAN
—— BCP	7	28	35	18.0
----- AP	6	22	28	24.0

FIGURE 2.—Duration of initial response according to induction regimen.

TABLE 3.—Degree of hematologic toxicity by treatment and risk status

Risk status	Treatment	No. of patients (%) by degree of toxicity	
		None to moderate	Severe
Good	BCP	50 (85)	9 (15)
	AP	37 (77)	11 (23)
Poor	BCP	23 (82)	5 (18)
	AP	17 (55)	15 (45)

might have had far-advanced disease; therefore, crossover data are not analogous to data from the use of sequential or concomitant alkylating therapy. On this point, Case et al (14) have reported that the combination of BCNU, cyclophosphamide, vincristine, melphalan, and prednisone is significantly more effective in remission induction without producing excessive toxicity. A randomized trial of what is still considered to be standard therapy (AP) versus the concomitant use of alkylating treatment has been initiated to address the value of combined alkylating agent therapy in myeloma.

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Randomized Study Comparing Doxorubicin, Cyclophosphamide, Vincristine, Methotrexate With Leucovorin Rescue, and Cytarabine (ACOMLA) With Cyclophosphamide, Doxorubicin, Vincristine, Prednisone, and Bleomycin (CHOP-B) in the Treatment of Diffuse Histiocytic Lymphoma[1,2]

Lee N. Newcomer, Ed C. Cadman,* Michael I. Nerenberg, Michael Chen, Joseph R. Bertino, Leonard R. Farber, and Leonard R. Prosnitz[3,4]

SUMMARY

Two combination chemotherapy programs, ACOMLA (doxorubicin, cyclophosphamide, vincristine, methotrexate with leucovorin rescue, and cytarabine) and CHOP-B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin), were evaluated in 29 prospective randomized patients with advanced diffuse histiocytic lymphoma. A complete response was achieved in 13 of the 15 patients (87%) treated with CHOP-B and in nine of the 14 patients (64%) treated with ACOMLA. The overall complete response rate was 75%. Two patients treated with ACOMLA and none of the CHOP-B-treated patients have relapsed. Median followup is 32 months for ACOMLA patients and 26 months for CHOP-B patients. Actuarial freedom from relapse at 2 years is 49.9% for ACOMLA and 93.3% for CHOP-B (*P* = 0.04). Toxicity was substantial, with eight nonfatal episodes of sepsis and three drug-related deaths. There have been no central nervous system relapses. Although patients treated with CHOP-B have a better response rate, the small numbers of patients treated to date preclude definitive conclusions.

[Cancer Treat Rep 66:1279–1284, 1982]

Diffuse histiocytic lymphoma is a potentially curable malignancy. Several regimens have produced complete response rates from 41% to 69% including C-MOPP (cyclophosphamide, mechlorethamine, vincristine, procarbazine, and prednisone); CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone); and CHOP-B (cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin) (1–3). Occasional modifications of these drug schedules have also been successful (4).

In 1972, Levitt et al (5) retrospectively evaluated 15 patients with reticulum cell sarcoma treated with COMLA (cyclophosphamide, vincristine, metho-

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[1] Received Sept 3, 1981; revised Dec 22, 1981; accepted Jan 7, 1982.

[2] Supported by Public Health Service grants CA-16539, CA-08341, and CA-09200 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. L. N. Newcomer is a clinical fellow of the American Cancer Society, J. R. Bertino is an American Cancer Society Professor, and E. C. Cadman is the recipient of a Faculty Research Award from the American Cancer Society. Part of this work was performed by M. I. Nerenberg as a thesis requirement for his doctoral of medicine degree.

[3] Departments of Internal Medicine (*L. N. Newcomer, E. C. Cadman, M. I. Nerenberg, J. R. Bertino,* and *L. R. Farber*), Pharmacology (*E. C. Cadman* and *J. R. Bertino*), and Radiation Therapy (*L. R. Prosnitz* and *M. Chen*), Yale University School of Medicine, New Haven, CT.

[4] We thank Carol S. Portlock, MD, for manuscript review, Diane Fischer for statistical evaluation, and Hallary Raeffer for manuscript preparation.

**Reprint requests to:* Ed C. Cadman, MD, Department of Internal Medicine, Section of Medical Oncology, Yale University School of Medicine, 333 Cedar St, PO Box 3333, New Haven, CT 06510.

trexate with leucovorin rescue, and cytarabine). Nine patients had a complete response. COMLA employed antimetabolites when cells theoretically would have been in logarithmic phase growth following the high fraction cell kill from cyclophosphamide and vincristine. More recent laboratory data have indicated a dose- and schedule-dependent synergism between methotrexate and cytarabine in L1210 leukemia cells and human myeloblasts (6).

We have previously reported our results using this drug program to treat 30 patients with diffuse histiocytic lymphoma. Twelve patients had a complete response, but six of these patients relapsed (7). A recent series of 42 patients treated at the University of Chicago with COMLA had better results; there were 20 (48%) durable complete remissions (8).

In 1975, we modified the COMLA regimen by adding doxorubicin, an efficacious agent for the treatment of diffuse histiocytic lymphoma (2–4). A randomized trial in stage III and IV diffuse histiocytic lymphoma was initiated to compare this new antimetabolite program, ACOMLA, to the commonly used drug combination CHOP-B.

PATIENTS AND METHODS

Between May 1976 and September 1980, 29 consecutive patients with advanced stage (III or IV) diffuse histiocytic lymphoma were entered in the protocol after obtaining informed consent. Pathology was reviewed at our institution. Patients were not eligible if they had received prior chemotherapy. Prior radiotherapy did not exclude patients from the protocol. Measurable disease, an absolute granulocyte count $> 500/\text{mm}^3$, a platelet count $> 50{,}000/\text{mm}^3$, and age > 16 years were required for eligibility. Radiotherapy was used to treat only life-threatening sites of disease, and patients were excluded from the study if all the known sites of disease had received radiation. No patients were excluded for medical conditions or a poor Karnofsky status.

All patients were clinically staged using the Ann Arbor classification. Evaluation included cbc, liver and renal function tests, chest X-ray, bilateral bone marrow biopsies and aspirates, lymphangiography with iv pyelogram, gallium scan, and liver-spleen scans. Abdominal ultrasound, computerized axial tomography, and gastrointestinal series were obtained in patients with intra-abdominal involvement or gastrointestinal symptoms. All patients who had bone marrow involvement had a lumbar puncture for cytologic examination.

Those patients with stage III and IV disease were randomized to one of two treatments: ACOMLA or CHOP-B. ACOMLA consisted of cyclophosphamide ($1.0\ \text{g/m}^2$) and doxorubicin (hydroxydaunorubicin) ($40\ \text{mg/m}^2$) given on Day 1; vincristine (2.0 mg) given on Days 1, 8, and 15; and methotrexate ($120\ \text{mg/m}^2$) followed by cytarabine (cytosine arabinoside) ($300\ \text{mg/m}^2$) given 1 hour later on Days 22, 29, 36, 43, 50, 57, 64, 71 (fig 1). Leucovorin (25 mg) was administered orally 24 hours after the methotrexate and was continued every 6 hours for six doses. Three cycles, each lasting 3 months, were administered. CHOP-B consisted of cyclophosphamide ($1.0\ \text{g/m}^2$), doxorubicin ($40\ \text{mg/m}^2$), and vincristine (2.0 mg) given on Day 1; bleomycin (15 units) given on Days 1 and 5; and prednisone ($100\ \text{mg/m}^2$) given daily for the first 5 days. The cycle was repeated every 4 weeks, for a total of nine cycles. The total treatment time for both drug programs was 9 months. Drug dose was reduced by 33% if the wbc count was $< 2500/\text{mm}^3$ during the methotrexate and cytarabine phase of ACOMLA. If the nadir granulocyte count was $< 300/\text{mm}^3$ after cyclophosphamide and doxorubicin for either regimen, the subsequent drug dose was reduced by 33%.

One month after completion of therapy all patients were restaged using the same clinical procedures described for initial evaluation. Previous sites of disease which were clinically detectable were rebiopsied. Patients were followed regularly at 2-month intervals until 24 months after completion of therapy. Followup intervals were then increased to between 4 and 6 months. Complete response was defined as the total disappearance of all known disease for a duration of 2 months. Partial response was defined as a 50% reduction in tumor parameters for > 2 months.

Survival and freedom from relapse are calculated from the date of protocol entry and the date of response, respectively, using the Kaplan-Meier method. The curves were compared using the Wilcoxon test.

RESULTS

The clinical characteristics are summarized in table 1. Sites of involvement in the stage IV patients included gastrointestinal tract (four patients), lung (four), bone (three), bone marrow (three), pleura (two), and liver, pancreas, abdominal peritoneum, prostate, and breast (one each).

All patients achieving a complete response were clinically free of disease within 2 months of treatment initiation. Overall, 75% of the patients achieved a complete remission. Thirteen of the 15 patients treated with CHOP-B achieved a complete remission (87%). None of these patients has relapsed. Of

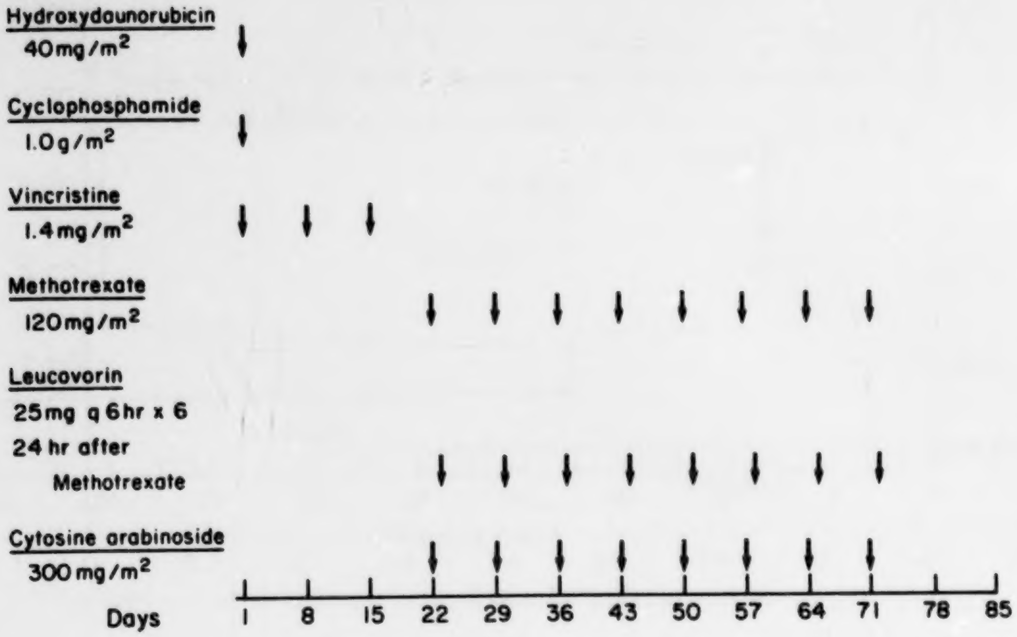
Hydroxydaunorubicin
40 mg/m2
Cyclophosphamide
1.0 g/m2
Vincristine
1.4 mg/m2
Methotrexate
120 mg/m2
Leucovorin
25 mg q 6 hr x 6
24 hr after
Methotrexate
Cytosine arabinoside
300 mg/m2
Days
1
8
15
22
29
36
43
50
57
64
71
78
85

FIGURE 1.—ACOMLA drug regimen schedule.

TABLE 1.—Clinical characteristics of patients with diffuse histiocytic lymphoma: ACOMLA vs CHOP-B

	ACOMLA group*		CHOP-B group†	
	No. (%) of patients	No. (%) with complete response	No. (%) of patients	No. (%) with complete response
Total	14	9 (64)	15	13 (87)
Stage				
IIIA	2 (14)	2 (100)	5 (33)	5 (100)
IIIB	1 (7)	0	0	—
IVA	8 (57)	5 (63)	7 (46)	6 (86)
IVB	3 (21)	2 (66)	3 (20)	2 (66)
Gastrointestinal involvement	3 (21)	1 (33)	3 (20)	3 (100)
Bone marrow involvement	3 (21)	0	0	—
Tumor > 10 cm	2 (14)	1 (50)	3 (20)	2 (66)
Concomitant radiation therapy	6 (43)	4 (66)	6 (40)	5 (83)

*Mean age: 57 ± 17.5 yrs.
†Mean age: 59 ± 12.6 yrs.

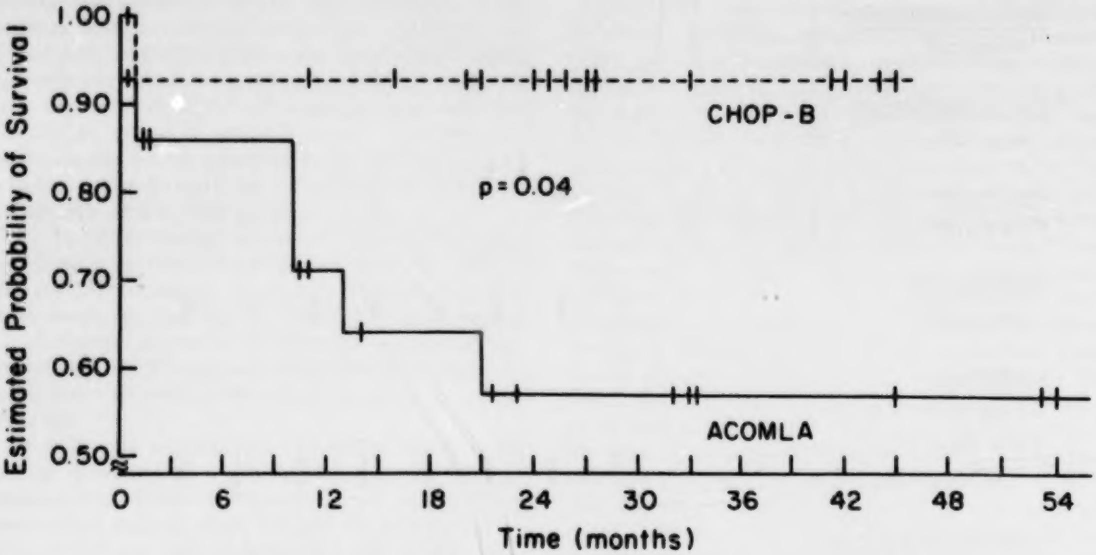

FIGURE 2.—Survival of patients with diffuse histiocytic lymphoma treated with ACOMLA or CHOP-B.

the 14 patients treated with ACOMLA, nine have had complete response (64%); two of these patients subsequently relapsed at 20 and 13 months. The difference between the percent complete remission was not significant ($P = 0.2$). One relapsing patient has achieved a second complete remission to CHOP-B. The average survival for the seven remaining patients who did not respond to therapy was 8 months. The number of patients is too small to accurately evaluate prognostic indicators. However, none of the three patients with bone marrow involvement achieved a complete response. Relapse in the central nervous system has not been documented in any patient. The survival and freedom from relapse curves are shown in figures 2 and 3, respectively.

Drug toxicity was substantial. Three drug-related deaths from sepsis occurred during induction therapy; two patients had received ACOMLA. Of the three patients dying from sepsis, one had gastric involvement with lymphoma, developed a perforation 7 days after his first drug treatment, and died from sepsis and acute respiratory distress syndrome 1 week later; the other two patients had extensive stage IV disease and a Karnofsky status of 10. None of these patients had bone marrow involvement or prior radiation therapy. Eight febrile episodes occurred during the first course of chemotherapy at approximately 10–14 days: four in each treatment arm. In all cases, the wbc count was $< 1000/\text{mm}^3$. The platelet count was never $< 75{,}000/\text{mm}^3$. There were no pulmonary complications associated with bleomycin. One patient developed congestive heart failure and angina pectoris while receiving CHOP-B, at a total doxorubicin dose of 240 mg/m^2. Nausea and vomiting were experienced by all patients during methotrexate and cytarabine administration. Cumulative doses of cyclophosphamide and doxorubicin were reduced by 50% in four patients receiving CHOP-B; only one patient was an induction failure. The dose of these drugs was also reduced by 50% in four patients treated with ACOMLA; none of these patients were induction failures. There was no hematologic toxicity following the methotrexate and cytarabine treatment.

Three patients developed second malignancies. Squamous cell carcinoma of the lung and prostatic carcinoma were discovered in two elderly patients treated with CHOP-B within 1 year of achieving a complete remission. Both are alive and free of lymphoma. Acute myelogenous leukemia was diagnosed 1 month after completion of ACOMLA in a patient who had a marrow involvement with diffuse histiocytic lymphoma at entry in the study. Re-evaluation of the pathology confirmed this unusual simultaneous occurrence.

Radiotherapy, which was never given to all sites of disease in any patient, was employed in various doses for potentially catastrophic lesions such as involved gastrointestinal tract or weight-bearing

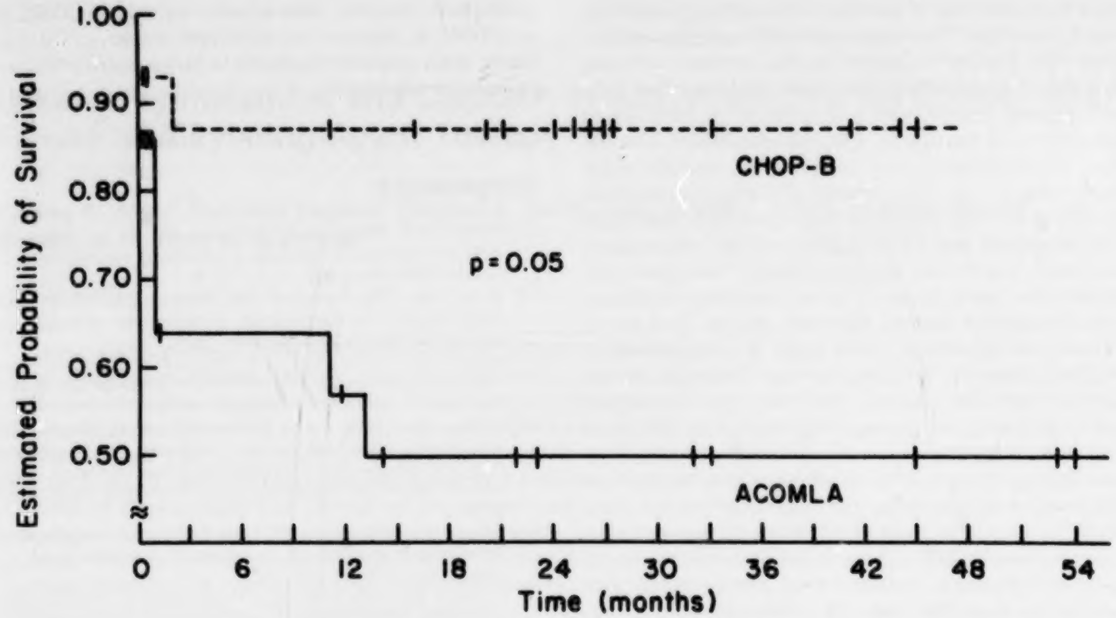

FIGURE 3.—Disease-free survival of patients with diffuse histiocytic lymphoma treated with ACOMLA or CHOP-B.

bones. The radiation therapy generally preceded drug treatment by 1–3 weeks, with an average of 2000 rads. Radiotherapy was utilized in six patients treated with CHOP-B (stomach, two; pelvis, two; and spleen and colon, one each). Five of these patients subsequently had a complete response to the chemotherapy. However, seven of the remaining eight patients treated with CHOP-B who were not given radiotherapy also achieved a complete response. In the ACOMLA arm, six patients were given radiotherapy (femur, two; pelvis, small bowel, stomach, and testicle, one each). Four of these patients achieved a complete response while only three of the other eight patients treated with ACOMLA who did not receive radiotherapy had a complete response. These differences are not statistically significant.

DISCUSSION

The treatment of advanced diffuse histiocytic lymphoma has improved significantly in the past 10 years. Early combinations of low-dose cyclophosphamide, vincristine, and prednisone (CVP) produced a complete remission rate of only 15% (9). The use of high doses of cyclophosphamide and the addition of doxorubicin appeared to increase the complete remission rate. Combinations including C-MOPP, CHOP, HOP (doxorubicin, vincristine, and prednisone), CHOP-B, and BACOP have produced complete remission rates of 41%–69% (1–4). Sweet and Golomb (10), in a recent review of the treatment of diffuse histiocytic lymphoma, note that patients who are carefully restaged clinically and remain relapse-free for at least 2 years appear to be cured of their disease.

The COMLA regimen evolved for several theoretic advantages. The antimetabolites, methotrexate and cytarabine, which kill cells that are synthesizing DNA, were given after tumor lysis from established agents to ensure maximal cell kill from these agents while the tumor was in a rebound of rapid growth. Sequential administration of cytarabine after methotrexate was also shown to kill cells synergistically by enhancing intracellular accumulation of cytarabine (6). In addition, both methotrexate and cytarabine cross the blood-brain barrier and therefore might possibly reduce relapses within the central nervous system. Intracranially transplanted L1210 leukemia will, for example, respond to systemically administered cytarabine in mice (11).

Sweet et al (8) recently reported that among 42 patients carefully restaged there were 23 complete responses and only three relapses. The median disease-free survival of patients in complete remission was 18 months. The median survival was 33 months. Only one patient relapsed in the central nervous system. A recent study by Skarin and Canellos (12), which used methotrexate (3 g/m^2 iv) in addition to bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (M-BACOD), was also quite successful in the treatment of diffuse histiocytic lymphoma. The complete response rate was 80% (45 responses among 56 patients). Seven patients relapsed, one in the central system. Two other patients who were apparently in complete remission also developed central nervous system lymphoma before the scheduled tenth cycle of drug administration. None of our patients had a relapse in the central nervous system. The contrast in central nervous system relapse between the study of Sweet and our study compared to the study of Skarin is interesting. Could it be that the cytarabine is the drug which accounts for this difference? In two separate evaluations from the National Cancer Institute (Bethesda, MD), the percent of central nervous system relapses in patients with diffuse histiocytic lymphoma was 29% and 12% (13,14).

Although patients treated with CHOP-B had a higher complete response rate, the differences in patient populations prevent definitive conclusions. Poor prognostic indicators which have been identified include bone marrow, gastrointestinal tract, or central nervous system involvement, the presence of B symptoms, or a tumor > 10 cm in diameter (15,16). The CHOP-B group is biased for a more favorable response by more stage III patients and an absence of patients with bone marrow involvement.

Drug doses differed in both treatment groups compared to other reported combinations. CHOP-B in the M. D. Anderson Hospital experience utilized 750 mg/m^2 of cyclophosphamide (3). The higher dose in our program may account for our higher response rate. The COMLA combination at both the University of Chicago and Yale University used 1.5 g/m^2 of cyclophosphamide (7,8). The addition of doxorubicin (40 g/m^2) to COMLA with cyclophosphamide dose reduction (1 g/m^2) does not appear to offer any advantage when compared to the University of Chicago series which did not use doxorubicin. Patients in the CHOP-B arm received three times more total doxorubicin (360 mg/m^2), cyclophosphamide (9 g/m^2), and vincristine (54 mg) than the ACOMLA patients. Because the differences between these two treatments were small, it is difficult to interpret the importance of the dose reduction in the ACOMLA program.

Both drug combinations have resulted in similar responses. CHOP-B is easier to administer with fewer clinic visits and less nausea and vomiting. ACOMLA or COMLA remains an excellent regimen for patients with contraindications to large doses of doxorubicin or bleomycin.

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Hexamethylmelamine and Cisplatin in Advanced Ovarian Cancer After Failure of Alkylating-Agent Therapy[1,2]

Steven E. Vogl,* Marcello Pagano, Thomas E. Davis, Nina Einhorn, Josh C. Tunca, Barry H. Kaplan, and James C. Arseneau[2]

SUMMARY

Thirty-eight women with advanced ovarian cancer resistant to alkylating-agent chemotherapy were treated with a combination of hexamethylmelamine and cisplatin. Hematologic toxicity was severe or life-threatening in seven of 13 patients treated with cisplatin at a dose of 100 mg/m^2 but was acceptable in 25 patients treated at 75 mg/m^2. Thirty-four percent of the combined group developed peripheral neuropathy and only 8% of those treated at 75 mg/m^2 developed transient azotemia. Nine complete responses and 12 partial remissions were noted among 38 patients treated, with no obvious advantage for the higher dose of cisplatin. The median duration of remission was 8 months and the overall median survival was 9 months. The response rate of 55% is approximately twice that reported with either agent given alone in similar patient populations and is equal to or better than that reported for more complicated regimens containing these drugs together with other, less active drugs.

[Cancer Treat Rep 66:1285–1290, 1982]

Hexamethylmelamine (HXM) (1–3) and cisplatin (DDP) (4–6) are two of the most active drugs in chemotherapy for ovarian cancer. These are the only drugs with objective response rates > 10% after failure of alkylating-agent chemotherapy (7). The use of these agents in combination is attractive since their major toxic effects differ. The major toxicity of HXM is neurologic whereas that of DDP is renal. Neither drug causes major myelosuppression at usual therapeutic doses. Exacerbation of the moderately severe vomiting induced by DDP can be avoided by delaying the start and limiting the dose of HXM (8). The present trial was a pilot study to identify the maximum doses of these agents which can be safely administered together after failure of alkylating-agent therapy for advanced ovarian cancer and to determine whether the use of the two drugs together may be better than either given alone.

A preliminary study at our institutions showed that the combination of these two drugs with doxorubicin (Adriamycin) was active and tolerable in this patient population, but that no patient was cured by such treatment (8). The response rate with this regimen, which we called "HAD," was 54%, with a median remission duration of 6 months. Doxorubicin was initially included because of evidence that its activity is equal to that of melphalan in patients without prior chemotherapy (9). Melphalan had become the standard alkylating agent used in the US because it is orally administered and produces neither vomiting nor alopecia. Because of new information that doxorubicin has little activity after failure of alkylating agents in ovarian cancer (7,10,11),

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[1] Received Sept 3, 1981; revised Jan 13, 1982; accepted Feb 2, 1982.

[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Chemotherapy Foundation and the New York City Division of the American Cancer Society. The following institutions participated in the study: Albert Einstein College of Medicine, Bronx, NY (CA-14958 and RR-00050; *S. E. Vogl* and *B. H. Kaplan*); Sidney Farber Cancer Institute, Boston, MA (CA-23318; *M. Pagano*); Wisconsin Clinical Cancer Center, Madison (CA-21076; *T. E. Davis* and *J. C. Tunca*); The Radiumhemmet-Karolinska Hospital, Stockholm, Sweden (*N. Einhorn*); and the University of Rochester Cancer Center, NY (CA-11083; *J. C. Arseneau*).

**Reprint requests to:* Steven E. Vogl, MD, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Ave, Bronx, NY 10461.

it seemed likely that a combination including only HXM and DDP would be as useful as HAD in such patients. In addition, deletion of doxorubicin would avoid alopecia and reduce myelosuppression.

Having decided that the combination of HXM and DDP was of interest and of potential value, it still remained unclear how much DDP to administer. Data on the optimal DDP dose after failure of alkylating agents are conflicting. In the initial experience of Wiltshaw and Kroner (4), 30 mg/m^2 given once every 3 weeks was approximately as active as 30 mg/m^2/day for 3 days every 3 weeks. Both regimens had response rates of about 27%. Results at the National Cancer Institute (Bethesda, MD) using DDP as a single agent at a dose of 70 mg/m^2 (response rate, 29%) were not significantly better (3). However, in a later study, Wiltshaw et al (12) obtained a 52% response rate with a dose of 100 mg/m^2, including two responses among seven patients who had had disease progression with lower doses of DDP. Bruckner et al (13) observed three second responses to 120 mg/m^2 of DDP among six patients who had responded to 50 mg/m^2 and then failed, but only one response among 14 who had failed to respond to 50 mg/m^2. The duration of these responses was not stated, making it difficult to assess the value of the remissions.

Unfortunately, the optimal DDP dose when this drug is given as part of initial combination chemotherapy is also not clear. The only study addressing this problem is that of Ehrlich et al (14) in which approximately 80 patients received 50 mg/m^2 of doxorubicin and 750 mg/m^2 of cyclophosphamide monthly with DDP given at either 50 mg/m^2 once a month or 20 mg/m^2/day for 5 days once a month. There was no difference in response rate, response duration, or survival between the two groups, but the cumulative DDP dose was limited to 300 mg/m^2 in each group. Thus, any advantage that might have occurred by giving more DDP early may have been wiped out by giving no further DDP after the third chemotherapy cycle in the higher-dose treatment program.

The present trial was a pilot study to determine the maximum tolerated dose of DDP with HXM in a patient population with progressive ovarian cancer in spite of alkylating-agent therapy. Based on Wiltshaw's data (4,12), we expected to be able to administer 100 mg/m^2 of DDP together with HXM. We undertook the present trial to make sure that toxicity was not prohibitive, since experience with this dose in this patient population had been limited. The maximum dose of HXM was determined by gastrointestinal tolerance. In our experience, very few patients can tolerate > 200 mg/m^2 in divided doses daily, so this dose of HXM was chosen for the current trial (8).

MATERIALS AND METHODS

Eligibility was restricted to women with a histologically proved diagnosis of epithelial ovarian cancer. Tumor had to be beyond the pelvis (stage III or IV) or there had to be unresectable pelvic disease. Progressive tumor despite an adequate trial of alkylating-agent chemotherapy was required, as was duration > 3 weeks from last chemotherapy or irradiation, and tumor which could be measured or at least evaluated for response. Patients were excluded from the study if they had had prior chemotherapy with either HXM or DDP; if they had wbc counts $< 4000/\mu l$, platelet counts $< 100{,}000/\mu l$, or serum creatinine > 1.5 mg/dl; or if they were > 75 years old or bedridden 100% of the time (Eastern Cooperative Oncology Group [ECOG] performance status [PS] 4).[3]

The original protocol regimen (HD-100) stipulated the administration of 100 mg/m^2 of DDP iv on Day 1 with hydration and diuresis. This program began with an iv bolus of 40 mg of furosemide, followed by 2 liters of 5% dextrose in 0.45% saline with 10 mEq of KCl/liter infused over 2 hours. Then 12.5 g of mannitol and 40 mg of furosemide were given iv as successive boluses and 100 mg/m^2 of DDP was infused over 30 minutes. This was followed by an infusion of 500 ml/hour of 5% dextrose in 0.45% saline with 10 mEq of KCl/liter for 4 hours.

After the first 13 patients, the DDP dose was reduced to 75 mg/m^2 (HD-75) and a simpler program of hydration and diuresis, which is suitable for outpatient treatment, was used. DDP was then given 30 minutes after beginning a 2-liter infusion of 5% dextrose in 0.45% saline with 10 mEq of KCl/liter given over 2 hours. Furosemide (40 mg) was given as an iv bolus at the start of the infusion, and mannitol (12.5 g) followed by DDP (75 mg/m^2) were given as successive iv boluses 30 minutes after the start of the infusion.

HXM (200 mg/m^2/day) was given orally in divided doses for 14 days. Therapy was started 8 days after DDP to allow patients to recover from the vomiting induced by DDP before beginning a potentially nauseating oral drug such as HXM. HXM dose was adjusted downward if nausea occurred. Cycles were repeated every 21 days.

Doses were reduced by 33% if, on the day of planned iv drug administration, the wbc count was 3000–4000/μl or the platelet count was 75,000–100,000/μl. Treatment was withheld on a week-by-week basis if blood cell counts were below these levels. HXM was given regardless of blood cell counts unless, on Day 14 of a given cycle, the wbc count was $< 1500/\mu l$ or the platelet count was $< 40{,}000/\mu l$, in which case HXM was discontinued for the remainder of that cycle and all further chemotherapy doses were reduced to 75% of otherwise stipulated levels for the remainder of protocol chemotherapy for that patient.

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[3] ECOG PS: 0 = fully active; 1 = ambulatory with symptoms; 2 = bedridden < 50% of the day; 3 = bedridden > 50% of the day; and 4 = 100% bedridden.

Complete response was defined as the absence of any evidence of disease in a patient without symptoms or signs related to ovarian cancer. Partial response was defined as a $> 50\%$ decrease in the sum of the products of perpendicular diameters of measurable lesions without increase in the size of any lesion, without deterioration in performance status and without a $> 10\%$ decrease in dry body weight. When only evaluable disease (ill-defined pelvic masses, ascites, or pleural effusion) was present, an estimated 80% improvement was the criterion for partial remission. Progressive disease was defined as a $> 25\%$ increase in the sum of the products of perpendicular diameters of measurable lesions, the development of a new lesion, a > 1-point deterioration in PS on the ECOG scale,[3] or a $> 10\%$ weight loss.

Therapy was continued until evidence of progressive disease. Pathologic specimens were reviewed by pathologists at the individual cooperating institutions in this trial, and the findings were correlated with the results.

Patients

Forty-one patients were entered in the trial; three were excluded from further analysis. One of these patients had concurrent metastatic breast cancer. The second patient was lost to followup on Day 3 after receiving only DDP. The third patient never received HXM because of gastrointestinal hemorrhage unrelated to ovarian cancer or its treatment. Of the 38 evaluable patients, 71% were ambulatory (ECOG PS O or 1), 42% had received prior radiotherapy, 60% had been treated with other drugs in addition to alkylating agents, and 89% had undergone major surgical procedures for ovarian cancer. The median age was 56 years (range, 32–73). The median interval from diagnosis to entry in this study ranged from 1 to 156 months (median, 12.5). All patients had tumors at least 2 cm in diameter at entry. All patients except three were white. Sixty-three percent of the patients had had no significant weight loss in the 6 months prior to entry, while 16% had had a weight loss $> 10\%$. Extra-abdominal disease was present in 29%. The institutional pathologist coded 42% as having serous cystadenocarcinoma, 32% as having solid adenocarcinoma, 11% as having mucinous carcinoma, 8% as having endometrioid carcinoma, and 5% as having clear cell carcinoma. Tumor was coded as well-differentiated in 11%, moderately well-differentiated in 45%, and poorly differentiated in 32%; differentiation was not stated for 13%. Total abdominal hysterectomy and bilateral salpingo-oophorectomy had been accomplished in 63%. Prior chemotherapy had produced objective tumor regression in 29%, and tumor progression in 45%. Available information did not permit evaluation of response to prior chemotherapy in the remainder.

Disease was measurable on physical examination in 33 patients (chest film also showed measurable disease in one) and on ultrasonogram in one. Disease was "evaluable," but not measurable, on physical examination in the remaining four patients.

RESULTS

As shown in table 1, the toxicity of the initial schedule of 100 mg/m^2 of DDP followed by HXM was severe. The 31% rate of life-threatening thrombocytopenia (platelet count $< 25{,}000/\mu l$) combined with the 23% rate of severe thrombocytopenia (platelet count, 25,000–50,000/μl) was sufficient to make us modify the protocol after 13 patients were entered, so that all patients were subsequently given 75 mg/m^2 of DDP. As can be seen in table 1, hematologic toxicity was within generally acceptable limits at the lower dose of DDP. Three of 13 patients receiving the higher dose of DDP developed azotemia, with peak serum creatinine levels of 9.2 and 2.2 mg/dl in two and a peak BUN of 26 mg/dl in the third. The first of these patients was considered to have had life-threatening renal failure. She did survive this episode and lived a total of 6½ months from entry.

TABLE 1.—Toxicity

	HD-100 (13 patients)	HD-75 (25 patients)
Platelet count (cells/µl)		
< 25,000	31%	8%
25,000–50,000	23%	24%
Wbc count (cells/µl)		
< 1000	0	0
1000–2000	0	28%
Required transfusion	38%	29%
Serum creatinine (mg/dl) > 1.5	23%	8%

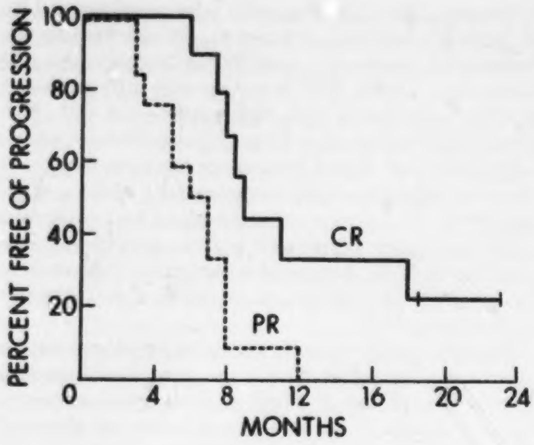

FIGURE 1.—Time to disease progression for partial and complete responders. Patients still in remission are indicated by vertical lines. Time of remission is measured from date of entry.

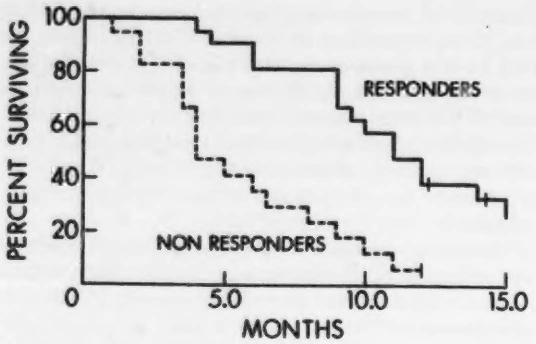

FIGURE 2.—Survival from date of entry as a function of response. Patients surviving at time of analysis are indicated by vertical lines.

In contrast to the high rate of azotemia seen at 100 mg/m^2 of DDP, only two patients (8%) who received 75 mg/m^2 developed transient increases of the serum creatinine to peak levels of 1.3 and 2.9 mg/dl. Renal function recovered promptly in both.

Hearing loss was noted in seven patients (18%) and was functionally significant in daily life for two of these. Thirteen patients (34%) developed peripheral neuropathy consisting largely of paresthesias, but six (16%) had functional limitation, with clumsiness, weakness, and poor balance in five and severe tremors in one.

Objective complete or partial remission of tumor was noted in seven of 13 patients receiving HD-100 (54%) and in 14 of 25 receiving HD-75 (56%). Nine of the 21 objective responses were complete by clinical and roentgenographic evaluation. Only one of four patients without measurable disease responded (partial remission, 3 months). The median duration of remission was 8 months (range, 3–23+). As shown in figure 1, the median duration of partial remission was 6.5 versus 9 months for clinical complete remission. As shown in figure 2, the median survival for patients with objective remissions was 10.8 months, significantly longer than that for those who did not respond (3.9 months; $P = 0.0006$, log-rank test).

Table 2 is an analysis of response rates by entry characteristics. None of the variables examined had significant influence on the rate of objective regression. In contrast, table 3 shows that the response rate for the 12 poorly differentiated tumors by Broders' criteria (nine of which were coded as solid adenocarcinomas), was only 25%, significantly lower than that for the remainder of the patients ($P = 0.016$, Fisher's exact test).

Table 4 is an analysis of prognostic factors for survival. Three factors were marginally significant for predicting prolonged survival: serous histology ($P = 0.025$, log-rank test) (15), Broders' grade 1, 2, or 3 ($P = 0.03$), and largest known tumor diameter at entry < 5 cm ($P = 0.03$). When these three factors were included in a multivariant analysis (Cox model [16]), the joint significance was $P = 0.07$. The decreased level of significance in the Cox model relates to the correlation between the factors and the relatively small sample size.

CONCLUSIONS

The results of this study establish the maximum tolerated dose of DDP as 75 mg/m^2 when combined with HXM in the treatment of patients with advanced ovarian cancer progressive in spite of alkylating-agent chemotherapy. Although DDP has been considered only minimally myelosuppressive in the past, thrombocytopenia proved to be the dose-limiting toxic effect in this patient population with extensive prior myelosuppressive treatment.

The present study served as a pilot trial for a later, ongoing, comparative, randomized, prospective clinical trial comparing two doses of DDP (37.5 or 75 mg/m^2), each given with HXM. This comparative trial has been underway since February 1980 and should complete its patient accrual in 1982. The goal of this study is to determine whether the higher dose of DDP is necessary to improve response rate, quality of response, or duration of response.

The response rate noted in this study (55%) is

TABLE 2.—Response rates by entry characteristics*

Characteristic	Yes	No
Prior radiotherapy	50% (16)	59% (22)
Only 1 prior chemotherapy drug	64% (14)	50% (24)
PS 0 or 1	59% (27)	45% (11)
Age ≤ 56 yrs	58% (19)	53% (19)
Interval from diagnosis ≥ 14 mos	66% (18)	50% (20)
Weight loss < 5%	56% (25)	54% (13)
Coelomic involvement only	56% (27)	55% (11)
Bulk† (cm)		
≤ 5	64% (14)	48% (21)
> 10	56% (25)	50% (10)

*Values in parentheses = No. of patients.

†Largest diameter of known tumor at entry (unknown in 3 patients).

TABLE 3.—Response rates by histologic type and grade*

	Response rate (No. of patients)
Histologic type	
Serous cystadenocarcinoma*	75% (16)
Solid adenocarcinoma†	38% (13)
Mucinous	50% (4)
Endometroid	0 (3)
Clear cell	50% (2)
Tumor grade (Broders')	
Well-differentiated, grade 1	75% (4)
Moderately differentiated, grade 2 and 3	65% (17)
Poorly differentiated, grade 4	25% (12)
Unknown	80% (5)

*Based on evaluation of pathologist at institution in which patient was first evaluated; values in parentheses = No. of patients.

†9 of 13 patients with adenocarcinoma had poorly differentiated tumors.

equal to that previously noted with HAD (8). The results are considerably better than those previously reported with either HXM or DDP as single agents (1–6) and somewhat better than those reported for combinations of DDP with doxorubicin plus cyclophosphamide (17); DDP with doxorubicin, cyclophosphamide, and HXM (18); or DDP with doxorubicin, 5-FU, and HXM in similar patient populations (19). Since doxorubicin, 5-FU, and cyclophosphamide have response rates < 10% in patients with progressive disease receiving alkylating-agent therapy (7,10,11), it is not surprising that their use in combination therapy in this patient population increases toxicity without increasing response rate. In the present study, as in our previous study (8), we are unable to identify any prognostic factors for response except for histologic grade.

The high rate of peripheral neuropathy in the present trial is a matter of concern. The overall incidence of 34%, with 16% having functional limitation, is considerably higher than that observed in our prior trial with HXM plus 50 mg/m^2 of DDP (12% peripheral neuropathy without any instance of functional limitation) (8). The peripheral neuropathy is probably due in part to HXM, but the higher rate in the present study may be a result of a higher DDP dose. DDP administration alone has been reported to cause peripheral neuropathy in approximately 3% of patients (20). We have seen only one case of peripheral neuropathy in approximately 150 patients treated with DDP who did not receive concomitant vincristine or HXM (known neurotoxic agents) (21). Since a Southwest Oncology Group study of HXM in breast cancer suggested, but did not prove, that concomitant administration of pyridoxine (vitamin B_6) reduces the incidence and severity of peripheral neuropathy from HXM (22), one half of the patients in our randomized trial will receive prophylactic pyridoxine at a dose of 300 mg/day while they are receiving HXM and DDP.

Of 38 evaluable patients in the current trial, only two remain in clinical complete remission 18 and 23 months after entry. While it remains conceivable that one or both of these patients is cured, it is clear that reserving the administration of HXM and DDP until failure of alkylating-agent chemotherapy will not have substantial impact on the overall survival of women with advanced ovarian cancer. We have therefore advocated the use of these agents as part of initial chemotherapy, in which we have already shown them to substantially improve the rate of objective response as well as the quality of these responses (23,24).

TABLE 4.—Median survival in mos by entry characteristic*

	Yes	No
PS 0 or 1	8.5 (27)	7.3 (11)
Complete or partial remission	10.8 (21)	3.9 (17)
Serous histology	10.8 (16)	5.8 (22)
Broders' grade 1, 2, or 3†	9.8 (21)	5.5 (12)
Age ≤ 56 yrs	9.5 (19)	5.8 (19)
Bulk ≤ 5 cm‡	11.0 (14)	5.8 (21)
Weight loss < 5%	9.0 (25)	6.0 (13)
Interval from diagnosis > 12 mos	9.3 (19)	7.0 (19)
Prior TAH-BSO§	8.5 (17)	7.8 (21)
Coelomic involvement only	9.0 (27)	6.0 (11)
Prior single drug only	9.0 (14)	6.0 (24)

*Values in parentheses = No. of patients.

†Grade is unknown in 5 patients.

‡Largest diameter of known tumor at entry (unknown in 3 patients).

§Total abdominal hysterectomy-bilateral salpingo-oophorectomy.

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Chemotherapy Versus Chemoimmunotherapy With Levamisole or *Corynebacterium parvum* in Advanced Lung Cancer[1,2]

A. Philippe Chahinian,* Judith Goldberg, James F. Holland, Arlene Reisman, Ira S. Jaffrey, and Eugen M. Mandel[3,4]

SUMMARY

A total of 109 patients with advanced lung cancer, all cell types, were randomized between MACC chemotherapy only, consisting of methotrexate, doxorubicin (Adriamycin), cyclophosphamide, and lomustine (CCNU); MACC plus levamisole (LMS) orally; and MACC plus *Corynebacterium parvum* (CP) sc. Of these patients, 101 were evaluable, with no differences among the three treatment groups for overall response rate and survival time. Objective response rates and median survival times were 41% and 230 days for patients given MACC only, 39% and 257 days for those given MACC plus LMS, and 44% and 223 days for those given MACC plus CP, respectively. There was a significant increase in survival for patients with large cell anaplastic carcinoma receiving CP or LMS, particularly in the good-performance-status category. Pretreatment delayed cutaneous hypersensitivity to recall antigens in 50 patients had prognostic significance, but repeat tests after 2 months of treatment in 30 patients did not show different patterns of conversion among the three groups. There was no difference in hematologic toxicity among the three groups. With the possible exception of large cell anaplastic carcinoma, immunotherapy with LMS or CP as given in this trial does not appear to be therapeutically advantageous in advanced lung cancer.

[Cancer Treat Rep 66:1291–1297, 1982]

The results of chemotherapy for advanced lung cancer, particularly in cell types other than small cell anaplastic, have been disappointing. Despite combination chemotherapy, the overall 1-year survival generally remains < 10% (1). Only a few chemotherapeutic agents have shown some degree of activity. Following our results (2,3) with a four-drug combination, MACC, consisting of methotrexate, doxorubicin (Adriamycin), cyclophosphamide, and lomustine (CCNU), we decided to investigate the role of immunotherapy in addition to chemotherapy in advanced lung cancer. Immunotherapy is most effective in cases of minimal tumor burden. However, on the basis of previous trials and because of the lack of new effective chemotherapeutic agents, the present randomized trial was undertaken in patients with advanced lung cancer. Two immunotherapeutic agents were selected. When combined with chemotherapy, *Corynebacterium parvum* (CP) treatment has produced increased survival but not increased response rates in three randomized trials in advanced lung cancer (4–6). Levamisole (LMS), when used as an adjuvant after surgery for lung cancer, was effective in delaying recurrence and

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[1] Received Oct 13, 1981; revised Dec 29, 1981; accepted Jan 18, 1982.

[2] Supported in part by Public Health Service grant CA-15936 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Departments of Neoplastic Diseases (*A. P. Chahinian, J. F. Holland, I. S. Jaffrey*, and *E. M. Mandel*) and Biomathematical Sciences (*J. Goldberg* and *A. Reisman*), Mount Sinai School of Medicine, City University of New York, NY.

[4] We thank Janssen Pharmaceutica, New Brunswick, NJ, for supplying levamisole, and Burroughs Wellcome Co, Research Triangle Park, NC, for supplying *Corynebacterium parvum*.

**Reprint requests to:* A. Philippe Chahinian, MD, Department of Neoplastic Diseases, Mount Sinai School of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029.

increasing survival, but only in patients weighing < 70 kg, allegedly because the dose of LMS was constant and not adjusted to weight or body surface in that trial (7). Clinical studies have suggested that these immunomodulators may restore delayed cutaneous hypersensitivity in cancer patients (4,8) and reduce hematologic toxicity from chemotherapy (4,9).

MATERIALS AND METHODS

Patient Population

A total of 109 consecutive patients with advanced, progressing, histologically proven bronchogenic carcinoma entered the study from March 1976 to March 1978. Cell types were categorized according to the World Health Organization's classification; all patients had advanced disease (stage III) (3). No patient should have received prior chemotherapy. Extent of disease and clinical workup have been defined previously (3). Performance status (PS) was evaluated according to the Cancer and Leukemia Group B scale: 0 = ambulatory without symptoms; 1 = ambulatory with symptoms; 2 = < 50% of the daytime in bed; 3 = > 50% of the daytime in bed; and 4 = bedridden.

Informed consent was obtained after the nature of the treatment had been fully explained. Patients were stratified within each of four major cell types (adenocarcinoma, squamous cell, large cell anaplastic, and small cell anaplastic) and were randomized among the three treatment groups. The first group received chemotherapy with the four-drug MACC combination, consisting of 30–40 mg/m^2 of methotrexate iv, 30–40 mg/m^2 of doxorubicin iv, 300–400 mg/m^2 of cyclophosphamide iv, and 30 mg/m^2 of lomustine orally (3). This regimen was repeated once every 3 weeks in the absence of residual toxicity. Peripheral blood cell counts were repeated every 10 days and the dose of chemotherapy was adjusted according to the nadir wbc and/or platelet counts. Doxorubicin was discontinued after a total dose of 550 mg/m^2. The second group received MACC and LMS (supplied in 50-mg tablets) at a dose of 2.5 mg/kg orally during 2 consecutive days every week. The third group received MACC and CP at a dose of 3.5 mg (0.5 ml) sc once a week, starting with chemotherapy. Each dose of CP was given at the root of one extremity by using a rotating schedule to minimize possible disability from local reaction. Criteria of response and duration of treatment have been defined previously (3).

Whenever a treatment delay ⩾ 48 hours was possible, delayed cutaneous hypersensitivity (DCH) skin tests were performed intradermally before the first treatment with five recall antigens including 5 units of purified protein derivative (Parke-Davis, Detroit, MI), 0.1 ml of mumps antigen (Eli Lilly and Co, Indianapolis, IN), 0.1 ml of a 1:100 dilution of Dermatophytin "O" (Hollister-Stier Labs, Spokane, WA), 0.1 ml of a 1:30 dilution of Dermatophytin (Hollister-Stier Labs), 40 units of streptokinase, and 10 units of streptodornase (Lederle Laboratories, Pearl River, NY). A positive test was defined by an induration ⩾ 5 mm at 48 hours.

Statistical Methods

The characteristics of patients randomized to the three groups were examined at the beginning of the study to determine whether the groups were comparable using methods of descriptive statistics, chi-square tests, and analysis of variance. Response rates overall and within strata defined by cell type were compared among treatment groups using chi-square tests. With 34 patients in each of any two treatment groups, an absolute difference of 35% from 30% in response rates would be detectable with a significance level of 10% (two-sided) and a power of 80%; a difference of 25% would be detectable with a power of 50%. Kaplan-Meier cumulative survival curves from date of first treatment (which was within days of randomization) were compared for the treatment groups using Breslow's extension of Gehan's generalized Wilcoxon test and the Mantel-Cox log-rank chi-square test (10). When results with both tests were similar, only log-rank values are given. Median survival times are shown for descriptive purposes only. Cox regression models for censored data were also used to further examine possible prognostic factors jointly in relation to treatment (11). All P values are given for two-tailed tests.

RESULTS

Of the 109 randomized patients, eight were unevaluable (four were lost to followup, two had prior chemotherapy with components of MACC, one had an inadequate dose of chemotherapy, and one had no measurable disease). The present analysis is therefore restricted to the remaining 101 evaluable patients, five of whom are still alive. Patient characteristics were comparable in the three groups (table 1). There was no difference in age distribution among the three arms: mean age of 57.3 years for MACC alone, 59.9 for MACC plus LMS, and 58.2 for MACC plus CP.

Response to Therapy

Response rates are shown in table 2. No significant differences were noted among the three groups

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%) treated with—		
	MACC only	MACC + LMS	MACC + CP
Evaluable patients	34 (100)	33 (100)	34 (100)
Sex			
Male	24 (71)	23 (70)	23 (68)
Female	10 (29)	10 (30)	11 (32)
PS			
0	9 (27)	10 (30)	6 (18)
1	11 (32)	15 (46)	16 (47)
2	9 (27)	7 (21)	7 (20)
3	4 (12)	1 (3)	5 (15)
4	1 —	0 —	0 —
Prior treatment			
Surgery	5 (15)	2 (6)	4 (12)
Radiotherapy (lung)	9 (26)	10 (30)	10 (29)
Extent of disease			
Limited	7 (21)	7 (21)	8 (24)
Extensive	27 (79)	26 (79)	26 (76)
Metastases			
Bone	15 (44)	16 (48)	11 (32)
Pleura	3 (9)	4 (12)	8 (24)
Lung	7 (21)	1 (3)	4 (12)
Liver	3 (9)	4 (12)	5 (15)
Brain	1 (3)	3 (9)	1 (3)
Soft tissue	3 (9)	1 (3)	1 (3)

overall nor within each cell type. The duration of therapy until disease progression ranged from a mean of 160 days for patients given MACC plus LMS to 198 days for patients given MACC plus CP. No major differences were seen when response rates among the three groups were compared within strata defined by patient characteristics (age, sex, extent of disease, cell type, and PS).

Survival

Overall survival (fig 1) was nearly identical among the groups for all evaluable patients (log-rank $\chi^2_2 = 0.3$; $P = 0.9$). These results were unchanged when the eight nonevaluable patients were included. The observed 1-year cumulative survival rate was 32% (SE = 8%) in each of the three treat-

TABLE 2.—% objective response by cell type*

Cell type	% response							
	MACC only		MACC + LMS		MACC + CP		Total	
	CR	PR	CR	PR	CR	PR	CR	PR
Adenocarcinoma	11	33	8	33	9	9	9	25
	(N = 9)		(N = 12)		(N = 11)		(N = 32)	
Squamous cell	0	20	0	33	20	30	8	27
	(N = 10)		(N = 6)		(N = 10)		(N = 26)	
Large cell anaplastic	0	33	0	50	12	50	5	45
	(N = 6)		(N = 6)		(N = 8)		(N = 20)	
Small cell anaplastic	44	22	33	0	60	0	43	9
	(N = 9)		(N = 9)		(N = 5)		(N = 23)	
Total	15	26	12	27	20	24	16	26
	(N = 34)		(N = 33)		(N = 34)		(N = 101)	

*Objective response includes complete response (CR)—the disappearance of all clinical, radiologic, and laboratory evidence of tumor, and partial response (PR)—a > 50% decrease in the sum of the product(s) of perpendicular diameters of measurable lesion(s) without the appearance of new lesion(s) elsewhere.

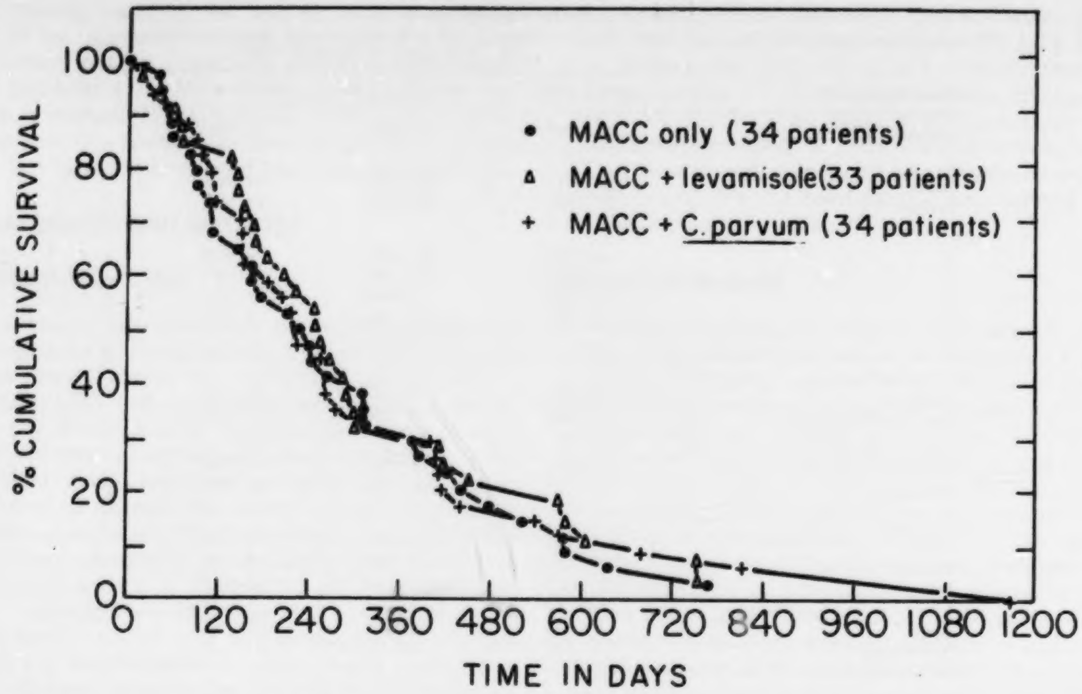

FIGURE 1.—Survival in days from 1st treatment of all 101 evaluable patients with advanced lung cancer, all cell types combined, randomized to receive MACC chemotherapy only, MACC + levamisole, or MACC + *C. parvum*. Survival curves are nearly identical among the 3 groups.

ment groups. Eight patients survived > 2 years (three with small cell, three with large cell, one with adenocarcinoma, and one with squamous cell). They were distributed among the three treatment groups and included five complete responders, two partial responders, and one patient with stable disease. Table 3 shows median survival times for subgroups of patients. Regardless of treatment group, the median survival of all 42 objective responders (419 days) was significantly longer than the median survival of all 59 nonresponders (155 days) (log-rank χ^2_1 = 33.1; $P < 0.005$). This difference remained significant within major PS categories (0 and 1 or ⩾ 2). For patients with a poor PS (⩾ 2), there was a suggestion of lengthened survival with immunotherapy (log-rank χ^2_2 = 6.7; P = 0.04).

There were no significant differences in survival among the three arms when sex, response to therapy, or extent of disease was considered.

Further multivariate analyses of survival using Cox regression models were carried out to determine whether any factors jointly affected prognosis in relation to treatment. When treatment, sex, age, PS, cell type (small cell vs non-small cell), and extent of disease (limited vs extensive) were included in the models, the observed mortality was not accounted for by these variables jointly (χ^2_2 = 6.65; $0.50 > P > 0.25$). Of these variables, only PS appeared to have some prognostic impact (coefficient/SE = 1.77; $0.05 < P < 0.10$) when adjusted for other variables.

Comparison of treatment groups within cell types indicated that the median survival for the few patients with large cell anaplastic carcinoma was significantly longer with MACC plus LMS or MACC plus CP compared to MACC only (log-rank χ^2_2 = 14.6; P = 0.001). This difference persisted when only patients with PS 0 or 1 were considered. No difference in survival among the three arms was seen in patients with small cell anaplastic carcinoma who responded, although their number was small.

Pretreatment DCH skin tests were performed on 50 patients distributed without bias among all treatment groups and cell types. Regardless of treatment group, median survival was 91 days for 12 patients with all skin tests negative and 287 days for 38 patients with at least one positive skin test (log-rank χ^2_2 = 16.1; P = 0.0001). There was no significant difference in survival with regard to skin tests among the three treatment groups, nor for

TABLE 3.—Median survival (days) by treatment group

	MACC only	MACC + LMS	MACC + CP
All patients	230	257	223
Sex			
All males	178	291	205
All females	242	171	404
Response to treatment			
Objective responders	426	419	407
Nonresponders	114	171	153
Extent of disease			
Limited	312	287	261
Extensive	230	251	155
PS			
0 or 1	478	250	227
⩾ 2	109	300	220
Cell type			
All except small cell	178	291	223
Adenocarcinoma	387	171	205
Squamous cell	166	291	189
Large cell anaplastic	114	419	407
Small cell anaplastic	316	208	257

one versus two or more positive tests. No apparent association was found between the number of positive pretreatment DCH skin tests and PS categories.

Toxicity

Toxicity was primarily hematologic, without significant differences between the three arms (table 4). The mean number of courses of chemotherapy needed to reach nadir wbc counts was 4.4 for MACC only, 4.5 for MACC plus LMS, and 4.7 for MACC plus CP. Similar figures for nadir platelet counts were 5.7, 4.4, and 3.2, respectively. None of these differences was significant. Mucositis occurred in 16 patients (seven given MACC only, three given MACC plus LMS, and six given MACC plus CP). Fever and infection during leukopenia were seen in seven patients. Cardiac toxicity included atrial flutter, atrial premature contractions, and decreased QRS volt-

TABLE 4.—Drug dose and hematologic toxicity

	MACC only	MACC + LMS	MACC + CP
No. of cycles of MACC			
Mean ± SE	8.0 ± 1.08	7.5 ± 1.01	8.0 ± 1.08
Range	1–24	1–23	1–25
Average drug dose (mg/m²)/patient/course			
Methotrexate	29.7	29.8	31.4
Doxorubicin	32.2	32.2	32.2
Cyclophosphamide	352.9	344.0	350.7
Lomustine	26.6	25.6	27.6
Wbc counts (cells/mm³)			
Nadir (mean ± SE) for all patients	3990 ± 740	3470 ± 370	4650 ± 710
< 3000 cells/mm³			
No. of patients	15	16	14
Median nadir	1100	1750	1750
Range	225–2900	400–2700	400–2700
Platelet counts (cells/mm³)			
Nadir (mean ± SE) × 10³ for all patients	163 ± 21	148 ± 15	197 ± 24
< 100,000 cells/mm³			
No. of patients	5	7	8
Median nadir × 10³	55	69	70
Range × 10³	20–92	10–90	10–98

age > 30% (one patient each). Only one patient developed congestive heart failure after receiving the full dose of doxorubicin. However, this patient responded to digoxin and diuretics. Hemorrhagic cystitis occurred only once. There were no significant hepatic or renal toxic effects. There was one treatment-related death from pneumonitis and fulminant septic shock during leukopenia in a patient receiving MACC plus LMS.

Immunotherapy was well-tolerated. Injections of CP were minimally painful, not requiring the use of local anesthetics, and resulted in slight to moderate local induration. A 25-mm ulceration occurred in only one patient. Two patients developed fever of 100°F–102°F after CP, and it was discontinued in one of them. Nine patients received CP every 3 weeks only, together with chemotherapy, because of the inconvenience of commuting weekly. Their response rate and survival were not different from patients receiving weekly CP. LMS was also well-tolerated. The most common side effect was a sensation of metallic taste (four patients). Nausea and vomiting occurred in one patient, and a maculopapular facial rash was observed in another. Agranulocytosis was seen in two patients. It was difficult to ascribe it to LMS, however, since all patients received chemotherapy concomitantly and since overall hematologic toxicity was not different among the three arms. One of these patients developed a leukopenia of 1500 cells/mm^3, with 2% neutrophils, 2% eosinophils, 23% monocytes, and 73% lymphocytes after the fifth cycle of MACC; there was also a mild thrombocytopenia of 122,000 cells/mm^3. Blood cell counts returned to normal within 5 weeks and no further hematologic toxicity occurred with the same dose of chemotherapy without LMS. The other patient developed a leukopenia of 1500 cells/mm^3 with 0% neutrophils, 3% eosinophils, 1% monocytes, and 96% lymphocytes (after the 11th cycle of MACC); there was also a thrombocytopenia of 80,000 cells/mm^3. Blood cell counts returned to normal within 3 weeks. No further hematologic toxicity was seen with the same dose of chemotherapy without LMS. This patient was rechallenged with LMS 2 months later without hematologic toxicity.

DISCUSSION

The present study extends our earlier reports of MACC chemotherapy in advanced lung cancer (2,3). The overall median survival of 33 weeks for patients receiving MACC is very close to the 36 weeks previously reported in a pilot trial of 83 patients similarly treated (3). A recent trial of MACC by the Eastern Cooperative Oncology Group showed a low response rate and prohibitive toxic reactions in patients with squamous cell carcinoma and adenocarcinoma of the lung (12). Several factors that may have contributed to these discrepancies have been discussed (13). Cohen et al (14) reported a 23% response rate, with prolongation of survival without prohibitive toxic effects in 72 patients with advanced lung cancer treated with MACC at the lower dose range. Corkery et al (15) obtained a 22% response rate (35% in limited disease) in 40 patients with lung cancer (cell types other than small cell) treated with a modified MACC regimen given on Days 1 and 8 every 6 weeks. In an ongoing trial of the North Central Cancer Treatment Group, patients with advanced adenocarcinoma and large cell anaplastic carcinoma of the lung are randomized to receive chemotherapy with MACC or cyclophosphamide, doxorubicin, and cisplatin. Response rates are presently similar (35% and 33%, respectively), but gastrointestinal toxicity was more acceptable with MACC (16). In a randomized trial of 39 patients with advanced lung cancer (cell types other than small cell), median survival was 34.5 weeks for MACC compared to 8.5 weeks for a placebo (17).

The addition of LMS or CP as given in this randomized trial did not increase overall response rates or survival. There was no bone marrow protection from drug toxicity by either CP or LMS and no demonstrable effect on DCH skin test when compared to chemotherapy alone. Similarly, other investigators using various routes, doses, and timing of CP in relation to chemotherapy have failed to show an improvement in survival (18–21), drug-induced hematologic toxicity (18,20), or DCH skin tests (18) in patients with advanced lung cancer. The activity of LMS as an adjuvant to surgery in lung cancer has not been confirmed in two trials (22,23), and its effect on DCH skin tests has been questioned (24).

Results of multiple significance tests carried out within subgroups must be interpreted with caution. Analysis by cell type in the present trial showed a significant benefit for immunotherapy in patients with large cell anaplastic lung cancer. This was particularly true in patents with a good PS (0 or 1). On the other hand, for all lung cancer types except large cell anaplastic, immunotherapy did not appear to be therapeutically advantageous. The number of patients with large cell anaplastic lung cancer remains small and confirmation is required. These results suggest that there may be different effects of immunotherapy on lung cancer according to cell type and cast serious doubts on studies of "composite lung cancer" cases. Just as small cell anaplastic cancer has been removed from lumped considerations because of differential chemotherapeutic response, so too large cell anaplastic carcinoma may appropriately be removed for future studies from the group of "non-small cell" type because of differential host response.

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Treatment of Advanced Malignant Melanoma With Vinblastine, Bleomycin, and Cisplatin[1]

E. Bajetta, R. Rovej, R. Buzzoni, M. Vaglini, and G. Bonadonna[2,*]

SUMMARY

Thirty-two consecutive patients with metastatic malignant melanoma were treated with vinblastine, bleomycin, and cisplatin (VBD) chemotherapy. There were 29 evaluable patients; complete remission was observed in three and partial remission in five. No patient with visceral metastasis showed objective response. The 10-month survival rate of responders was significantly higher than that of nonresponders (57.1% vs 18.2%, *P* = 0.01); however, present results do not confirm that VBD is superior to other drug combinations for malignant melanoma.

[Cancer Treat Rep 66:1299–1302, 1982]

Dacarbazine (DTIC) is still the treatment of first choice for many patients with metastatic malignant melanoma and, irrespective of the dose schedule (1), provides objective response in 18%–25% of patients, particularly at the level of soft tissue involvement (2,3). DTIC has also been utilized in several drug combinations, with (4,5) and without (6,7) immunostimulants, but the response rate was not significantly improved. Therefore, it appears worthwhile to study new drugs (8,9) and new combinations to verify their efficacy in malignant melanoma.

In 1980, Nathanson et al (10) reported a 71% objective response rate in 14 evaluable patients, utilizing a new treatment schedule: vinblastine, bleomycin, and cisplatin (VBD). This combination has also shown objective response in patients with visceral disease.

At the Istituto Nazionale Tumori of Milan we have tested VBD chemotherapy in metastatic melanoma, and the experience achieved with this drug regimen is the subject of the present report.

PATIENTS AND METHODS

From August 1980 to January 1981, 32 consecutive patients with metastatic malignant melanoma were treated with VBD. The main patient characteristics are listed in table 1. Twenty-five of 32 patients presented with multiple sites of involvement.

TABLE 1.—Patient characteristics

Characteristic	No. of patients	(%)
Total entered	32	
Total evaluable	29	(90.6)
Males/females	16/13	
Median age in yrs (range): 52 (21–65)		
Previous chemotherapy	18	(62)
Disease extent		
Soft tissues	19	(65.5)
Viscera ± soft tissues	10	(34.5)
Dose of vinblastine		
6 mg/m^2	12	(41.3)
4 mg/m^2	17	(58.7)
Total not evaluable	3	(9.4)
Early death	1	
Inadequate treatment*	2	

*< 1 full cycle.

Patients aged > 70 years or with important chronic nonneoplastic disease or brain metastases were considered ineligible for this trial. Before starting therapy, it was required that patients have measurable parameters for drug response, a life expectancy ⩾ 6 weeks, and a Karnofsky performance status ⩾ 40. Eighteen patients (62%) were previously treated with chemotherapy: 13 with DTIC, and five with other drugs. No patient had had prior therapy with vinblastine, bleomycin, or cisplatin.

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[1] Received Dec 10, 1981; accepted Jan 28, 1982.

[2] Istituto Nazionale Tumori, Milan, Italy.

**Reprint requests to:* Gianni Bonadonna, MD, Istituto Nazionale Tumori, Via Venezian, 1, 20133 Milan, Italy.

TABLE 2.—Response to VBD in 29 evaluable patients

	No. of patients			
	Evaluable	CR + PR	CR	MR
Total	29	8 (27.6%)	3	10
Males	16	3	1	6
Females	13	5	2	4
Soft tissues	19	8 (42.1%)	3	4
Skin	9	4	—	4
Nodes	10	4	3	2
Viscera	10	0	0	6

Treatment was performed in the hospital ward. The drug schedule was as follows. Vinblastine (6 mg/m^2) was injected iv on Days 1 and 2, bleomycin (15 mg/m^2) was administered by 24-hour iv infusion from Day 1 to Day 5, and cisplatin[3] (50 mg/m^2) was given iv by 1-hour infusion on Day 5. In an attempt to minimize myelotoxicity, the dose of vinblastine was reduced to 4 mg/m^2 starting with the 16th patient. Cisplatin was administered in 250 ml of 5% glucose in water, followed by 1000 ml of 5% glucose solution and 500 ml of 0.9% saline solution with 20 mEq/liter of KCl. Following hydration, patients were advised to drink 1 liter of fluid during the daytime. Patients were discharged on the following day. The regimen was repeated every 3 weeks. Cycles were delayed 1 week in the presence of persisting myelosuppression. After this delay, if the wbc count was between 2500 and 3600/mm^3 and/or the platelet count was between 75,000 and 100,000/mm^3, 50% of the planned doses of cisplatin and vinblastine were given.

An adequate therapeutic trial was defined as one full cycle of therapy. Chemotherapy was continued until disease progression occurred, and in patients who achieved complete response, two additional treatment cycles were administered. The maximum number of cycles administered was five. When VBD therapy failed, patients were given DTIC if they had not been previously treated with this drug.

Pretreatment hemogram, liver chemistry, serum calcium, renal function tests, and appropriate roentgenograms were obtained in all patients. Patients were evaluated before each treatment cycle with physical examination, repeated blood studies, X-rays, and scans as needed. Complete response (CR) was defined as the complete disappearance of all neoplastic lesions for at least 1 month. Partial response (PR) was defined as a $>$ 50% decrease in the product of the two largest perpendicular diameters in all measurable lesions for a minimum of 1 month. A $<$ 50% decrease in the product of the two largest perpendicular diameters of all measurable lesions was defined as minor response (MR). A $\geqslant$ 25% increase in initial lesions was considered as disease progression, regardless of concomitant regression in other tumor sites or occurrence of new lesions.

RESULTS

Of the 32 patients admitted to the study, 29 are included in the analyses for response and survival. Three patients were not evaluable: one because of early death unrelated to treatment and two because of inadequate treatment (less than one full cycle). None of the patients refused to complete treatment. Two patients received only one cycle, ten received two cycles, and eight received three cycles, because the disease was rapidly progressing. Of 86 cycles administered, 14 (16.2%) were given without cisplatin because of leukopenia and/or thrombocytopenia on Day 5. In particular, this occurred in five of 30 cycles given to patients with visceral disease. As shown in table 2, CR occurred in three (10.4%) of 29 evaluable patients, PR in five (17.2%), and MR in ten (34.4%). Of patients achieving CR or PR (27.6%), there was no substantial difference between the percent who had had no previous treatment (10.3%) and the percent who had received prior chemotherapy (17.3%). The response rate was also unrelated to the dose of vinblastine. The majority of responses were documented after the first two treatment cycles.

All eight patients achieving CR or PR had metastatic disease only in the soft tissues. MR was observed in six patients with visceral involvement (lung, three; liver, three). The median duration of CR plus PR was 3 months (range, 1–10). Two patients who achieved CR are still alive, and one is still disease free after 10 months. With the exception of one patient, the site of relapse in responders occurred in previously involved sites.

Figure 1 shows that the survival rate of respond-

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[3] Provided by Bristol-Myers Co, International Division, New York, NY, and Farmitalia-Carlo Erba, Milan, Italy.

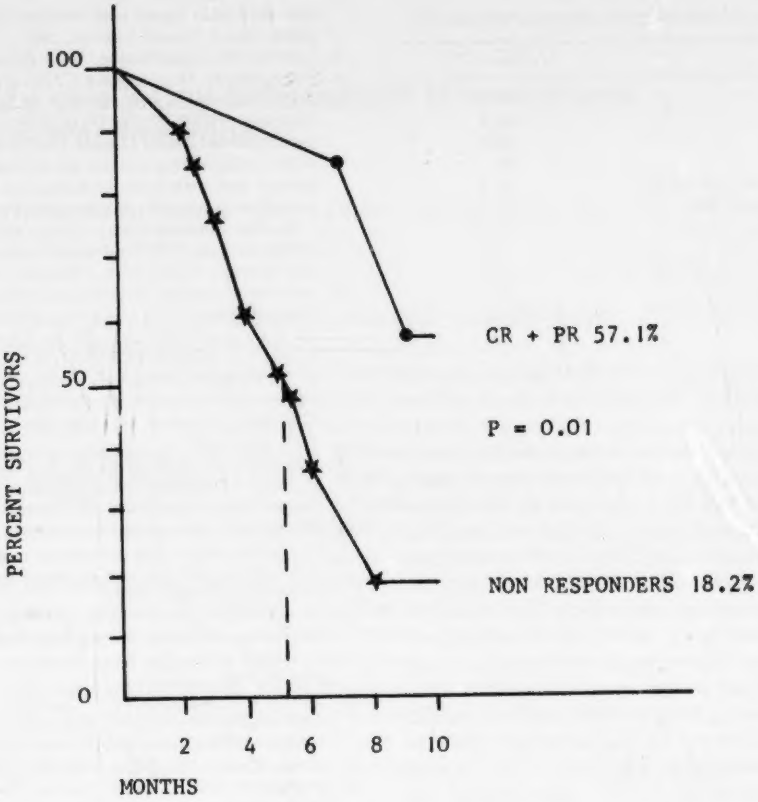

FIGURE 1.—Survival of responders.

ers was significantly higher than that of nonresponders (57.1% vs 18.2%, $P = 0.01$). The 10-month analysis from start of chemotherapy indicated that survival rate was significantly better for patients with soft tisue involvement than for patients with visceral deposits (39.1% vs 14.9%, $P < 0.02$). On the contrary, there was no significant difference in survival rates between patients previously treated or untreated with chemotherapy (25.7% vs 49%, $P = 0.39$) or between females and males (42.1% vs 27.3%, $P = 0.55$).

No drug-related deaths were observed. The incidence of side effects is shown in table 3. Fever, nausea, and severe vomiting were the most frequent toxic effects, while myelosuppression was the main treatment-limiting factor. Eleven patients had wbc counts $< 1000/\text{mm}^3$, 12 had platelet counts $< 60{,}000/\text{mm}^3$, and the nadir for both counts after each cycle occurred from Day 4 to Day 7. The decrease in wbc count appeared to be a cumulative toxic effect but the decrease in platelet count did not. None of the patients had renal abnormalities, and serum creatinine and blood urea nitrogen, in particular, always ranged within normal values. The incidence of side effects was about the same in previously untreated and treated patients.

CONCLUSION

Our prospective study failed to confirm the original data of Nathanson et al (10). In particular, no patient with visceral metastasis achieved objective response. VBD was as effective as other drug combinations recently tested in metastatic malignant melanoma (11–13), and considering the known limitation of cisplatin in terms of cumulative toxicity, VBD does not appear to be indicated for general use. More recently, Nathanson et al (14) have expanded their experience with VBD and reported an objective response in 43% of all patients. There were three CRs in patients with visceral disease and none in patients with soft tissue involvement. On the con-

Table 3.—Incidence of side effects in 29 evaluable patients

Side effect	Incidence (%)
Stomatitis	10.3
Alopecia	65.5
Nausea and vomiting	89.6
Fever	100
Leukocyte count $<$ 1000 cells/mm^3	34.4
Platelet count $<$ 60,000 cells/mm^3	41.3
Adynamic ileus	17.2
Skin changes	3.4
Bronchopneumonia	3.4
Herpes zoster	3.4

trary, we have observed three CRs in patients with soft tissue metastases and only MR in six of ten patients with visceral deposits. The observed differences in total response rates between our series and that of Nathanson et al could be due, in part, to the more aggressive schedule used by the American group: the dose of cisplatin was not modified in leukopenic patients on Day 5 (14). The difference in the aggressiveness of treatment was also reflected in the incidence of side effects (eg, abnormal pulmonary function tests and mild renal impairment), which were observed in the series of Nathanson et al and not in our own.

Our findings reemphasize that positive results utilizing a limited series of patients can often be misleading, as observed by Abele et al (15). In fact, with the combination of CCNU (lomustine), vincristine, and bleomycin, the Belgian group had initially observed an unusually high rate of 40%, which subsequently decreased to 25% when the total number of patients entered in the study became large.

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Phase I Study of a 5-Day Schedule of Mitoxantrone (Dihydroxyanthracenedione)[1,2]

William R. Wynert, Harold A. Harvey,* Allan Lipton, James Schweitzer, and Deborah S. White[3,4]

SUMMARY

Mitoxantrone (1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]-amino]-9,10-anthracenedione dihydrochloride) in animal studies appears to have a mechanism of action and broad antitumor spectrum similar to the anthracyclines and in preliminary animal studies is without cardiotoxicity. To determine the maximally tolerated dose, mitoxantrone was given to 25 patients with various advanced solid tumors. Sixteen patients had received prior doxorubicin and/or lomustine (CCNU). Myelosuppression was the major toxic effect. All 13 patients who received mitoxantrone doses ≥ 2.73 mg/m^2 × 5 days experienced moderate (2000–3000 cells/mm^3) or severe (< 2000 cells/mm^3) leukopenia. Thrombocytopenia was also encountered in three of these 13 patients (severe in one, $< 50{,}000$ cells/mm^3). At dose levels < 2.73 mg/m^2 × 5 days, myelosuppression was seen in four of 12 patients (three with mild leukopenia and one with mild thrombocytopenia). The blood cell count nadir occurred at 10 days and full recovery occurred by Day 21. Minor clinically insignificant ECG changes occurred in five patients. Minor and transient antitumor effects were seen in five patients. The maximum tolerated dose of mitoxantrone over a 5-day course is 2.73 mg/m^2, with leukopenia being the limiting toxic effect.

[Cancer Treat Rep 66:1303–1306, 1982]

Mitoxantrone (fig 1) belongs to the synthetic class known as the anthraquinones. Like doxorubicin, mitoxantrone binds to DNA, inhibiting nucleic acid synthesis (1,2).

Mitoxantrone shows activity in a wide range of experimental animal tumors. It is highly active against B16 melanoma, colon tumor 26, and P388 and L1210 leukemias. It showed moderate activity against P388/Adria and was ineffective when tested against Lewis lung carcinoma and Ridgway osteogenic sarcoma (3–5).

In contrast to the anthracyclines, preclinical studies have not demonstrated cardiotoxicity. In monkeys and dogs receiving mitoxantrone, no abnormalities were noted on auscultation, ECG, or blood pressure monitoring. Animals that were killed showed no cardiac pathology.[5] Ante- and post-mortem toxicity in animals has largely been confined to the gastrointestinal system and bone marrow. Because of this wide spectrum of antitumor activity and the apparent lack of cardiotoxicity, this anthracenedione appears to be an attractive candidate for clinical trials.

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[1] Received July 21, 1981; revised Jan 20, 1982; accepted Feb 16, 1982.

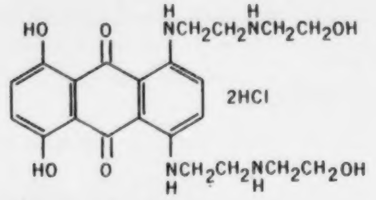
[2] Supported by the Medical Research Division, American Cyanamid Co, Lederle Laboratories, Pearl River, NY, and presented in part at the 1981 meeting of the American Society of Clinical Oncology.

[3] Department of Medicine, Division of Oncology, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey.

[4] We thank Mrs. Judy Weigel for her help in preparing this manuscript.

**Reprint requests to:* Harold A. Harvey, MD, Division of Oncology, The Milton S. Hershey Medical Center of The Pennsylvania State University, Hershey, PA 17033.

[5] Animal Studies of Cardiotoxicity With Mitoxantrone. Unpublished data on file in the Toxicology Research Section, Lederle Laboratories Division, American Cyanamid Co, Pearl River, NY.



1,4-Dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]-ethyl]amino]9,10-anthracenedione dihydrochloride

FIGURE 1.—Structure of mitoxantrone.

In a previous report, Von Hoff et al (6) treated 25 patients with a single iv dose every 4 weeks in a range of 1.2–14 mg/m^2 (6). We conducted a phase I clinical study of mitoxantrone using a schedule of daily doses for 5 consecutive days. The starting dose of 0.34 mg/m^2 was determined by taking two thirds of the "toxic dose low" in dog studies (0.515 mg/m^2/day for 5 days). The goals of this study were to establish the maximum tolerated dose of mitoxantrone administered on a 5-day schedule and to determine the clinically significant toxic effects of this agent. The maximum tolerated dose was defined as that dose which would consistently produce a grade 3 (or greater) toxicity of a clinically serious nature in most patients.

MATERIALS AND METHODS

Patient Selection

Mitoxantrone was given to 25 patients (median age, 56 yrs; range, 39–73) with a variety of incurable solid tumors (table 1). Nine patients had received prior radiotherapy and 16 had received chemotherapy (ten received doxorubicin, six received cyclophosphamide, and six received lomustine [CCNU]). Eight patients had received no prior chemotherapy since it was felt that there was no effective standard chemotherapy for their extent and types of tumor.

On initiation of mitoxantrone, all patients had a Karnofsky performance status $\geq$ 60% and had an expected survival $\geq$ 2 months. No patient had received cytotoxic chemotherapy within the preceding month. None had a history of cardiac disease or was receiving antiarrhythmic medication. All patients had a wbc count $>$ 4000/mm^3 and a platelet count $>$ 150,000/mm^3. Two of 25 patients had hemoglobins $<$ 10 g (one, 8 g and another, 9 g).

Written informed consent was obtained from all patients.

Treatment Schedule

Mitoxantrone was supplied by the Medical Research Division of the American Cyanamid Company as 0.5 mg/ml in 5- and 10-ml ampules. The drug was infused over 10–15 minutes in 250 ml of 5% dextrose in water on a daily × 5-day schedule monthly. The daily × 5-day dose level and number of patients at each level were as follows: 0.34 mg/m^2, four patients; 0.68 mg/m^2, three patients; 0.86 mg/m^2, two patients; 1.36 mg/m^2, three patients; 2.73 mg/m^2, five patients; 3.40 mg/m^2, six patients; and 4.00 mg/m^2, two patients. Patients were retreated at the same dose level every 4 weeks if they had experienced no toxicity, or at a lower dose level or not at all if severe toxicity had occurred with the first cycle.

Study Parameters

The following weekly tests were conducted with the first course of treatment: cbc with differential, platelet count, reticulocyte count, liver and renal function tests, electrolytes, glucose, calcium, phosphorus, CPK with MB fraction, ECG, and urinalysis. With subsequent courses, the cbc with differential and platelet count were repeated while the remainder of the above tests were performed monthly. Chest X-ray was obtained before each course. Patients were considered evaluable for toxicity if they received one dose of mitoxantrone. Standard response criteria were used to evaluate antitumor drug effect.

TABLE 1.—Types of solid tumors in 25 patients

Tumor type	No. of patients
Colorectal adenocarcinoma	11
Lung adenocarcinoma	4
Breast adenocarcinoma	2
Lung and larynx squamous cell carcinoma	1
Gastric adenocarcinoma	1
Melanoma	1
Lymphoma	1
Hypernephroma and perineal leiomyosarcoma	1
Hepatoma	1
Pelvic spindle cell sarcoma	1
Testicular embryonal carcinoma	1
Total	25

RESULTS

Over a 10-month period (concluded October 1980) 25 patients received a total of 54 courses of mitoxantrone. Twenty-three of the 25 patients are fully evaluable, having received at least one full course (daily single dose for 5 consecutive days). One patient at the 4.0-mg/m^2 dose level refused further therapy after receiving the drug for only 3 days but is considered evaluable for toxicity. Another patient received one full course of therapy but was only partially evaluable because of inadequate followup laboratory studies. Eight patients received single courses, ten had two courses, and the remainder had a range of three to six courses. None received concomitant antineoplastic medication.

Toxicity

Myelosuppression, especially leukopenia, was the major dose-limiting toxic reaction (table 2). All 13 patients receiving mitoxantrone at ≥ 2.73 mg/m^2 experienced moderate (2000–3000 cells/mm^3) or severe (< 2000 cells/mm^3) leukopenia and granulocytopenia. At dose levels < 2.73 mg/m^2, three patients experienced mild (3000–4500 cells/mm^3) leukopenia. One patient with extensive liver metastases developed severe leukopenia (< 1000 cells/mm^3) and thrombocytopenia (< 50,000 cells/mm^3) at 0.68 mg/m^2/day × 5 days.

Leukopenia, when it occurred, was seen with the initial course of mitoxantrone and recurred with subsequent courses at the same dose level. There did not appear to be any progressive decrease in the wbc count with repeated courses of therapy. Leukopenia appeared to be more prevalent in patients with extensive liver metastases. Three of four such patients experienced profound leukopenia (< 1000 cells/mm^3). The 5-day dose levels of these three patients were 0.68, 2.73, and 3.4 mg/m^2.

Thrombocytopenia was encountered in three of 13 patients receiving ≥ 2.73 mg/m^2 (table 3). One patient with metastatic melanoma involving the liver who was treated with a dose of 3.4 mg/m^2 experienced profound leukopenia (wbc count, 200/mm^3) and thrombocytopenia (platelet count, 29,000/mm^3). The only possible drug-related death occurred in this patient, who had cerebral metastases and developed intracranial hemorrhage on Day 15 while still thrombocytopenic. Mitoxantrone-related myelosuppression generally reached a nadir at Day 10, with full recovery by Day 21. A mild decrease in

TABLE 2.—Wbc toxicity*

Dose (mg/m^2 daily × 5 days)	No. of patients	No. of courses	No. of courses by wbc count nadir, in cells/mm^3 (grade)				
			> 4500 (0)	3000–4500 (1)	2000–3000 (2)	1000–2000 (3)	< 1000 (4)
0.34	4	6	6	0	0	0	0
0.68	3	3	1	1	0	0	1
0.86	2	4	3	1	0	0	0
1.36	3	16	8	4	1	3	0
2.73	5	10	2	1	1	2	4
3.40	6	12	3	1	1	3	4
4.00	2	3	1	0	1	0	1
Totals	25	54	24	8	4	8	10

*Mean nadir neutrophil count in patients with leukopenia was 412 cells/mm^3 (range, 16–1240).

TABLE 3.—Platelet toxicity

Dose (mg/m^2 daily × 5 days)	No. of patients	No. of courses	No. of courses by platelet count nadir, in cells/mm^3 (grade)				
			> 130,000 (0)	90,000–130,000 (1)	50,000–90,000 (2)	25,000–50,000 (3)	< 25,000 (4)
0.34	4	6	6	0	0	0	0
0.68	3	3	2	0	0	1	0
0.86	2	4	3	1	0	0	0
1.36	3	16	15	1	0	0	0
2.73	5	10	5	3	1	0	1
3.40	6	12	11	0	0	1	0
4.00	2	3	3	0	0	0	0
Totals	25	54	45	5	1	2	1

hemoglobin (1–2 g) was seen in 13 patients; however, this was probably a result of tumor progression rather than drug toxicity.

Nonhematologic Toxicity

Minor, clinically insignificant ECG changes were seen in five of 25 patients. These changes may have been related to mitoxantrone. Three of the five patients developed new, nonspecific ST-T wave changes, with two patients having such changes after the first cycle of mitoxantrone. A fourth patient developed new premature ventricular contractions. The premature ventricular contractions were noted 3 weeks after the second course of mitoxantrone and did not recur. A fifth patient experienced sinus tachycardia. Two of the above patients showing ST-T changes had received prior doxorubicin (one, a total of 300 mg/m^2). Radionuclide cardiac scanning was not performed in this study. There were no significant changes in serum CPK levels.

Other drug-related toxic reactions included mild nausea and vomiting in four patients and slight hair loss in three. No patient developed renal or liver dysfunction as a result of mitoxantrone therapy. There were no cases of phlebitis or toxicity to the skin or mucous membranes. Extravasation of small amounts of drug occurred in three patients but this produced no local tissue damage.

Antitumor Effects

In this phase I study involving 25 patients we observed modest objective antitumor effects only at dose levels of 3.4 mg/m^2. Four of eight patients receiving a 5-day course $\geqslant$ 3.4 mg/m^2/day showed an antitumor effect. There were two minor partial responses (< 50% shrinkage) and two disease stabilizations (one lung adenocarcinoma for 4 months and one colon carcinoma for 5 months). A patient with stage IV progressive, diffuse, poorly differentiated lymphocytic lymphoma showed a gratifying (but < 50%) tumor reduction in lymph node size after the first cycle of mitoxantrone. Unfortunately, he failed to return for further therapy. Another patient with pelvic spindle cell sarcoma had a minor partial regression lasting 3 months. Both of these patients had disease progression after prior extensive chemotherapy including doxorubicin. A final patient with epidermoid lung carcinoma, receiving 1.36 mg/m^2 over 5 days, showed disease stabilization of his lesion for 8 months.

DISCUSSION

The main goal of this study was to establish the maximum tolerated dose as defined above using the 5-day schedule. Although we were able to administer mitoxantrone to patients at dose levels of 3.4 and 4.0 mg/m^2 daily × 5 without grave complications, it became evident that serious leukopenia (grade 3; wbc count nadir < 2000/mm^3) had occurred in more than one half of the instances at the lower dose level of 2.73 mg/m^2 (table 2). We have therefore concluded that the maximum tolerated dose over a 5-day course in this study was 2.73 mg/m^2, with leukopenia being the limiting toxic reaction. Thrombocytopenia was infrequent. The nadir of the myelosuppression occurred at Day 10, with full recovery of the wbc and platelet counts by 2½–3 weeks. Leukopenia appeared to be more evident and pronounced in our patients with extensive liver disease or extensive prior chemotherapy or radiotherapy. Consideration of such factors may indicate a need for dose reduction and caution in administering mitoxantrone in future studies. Minor ECG changes of uncertain relationship to drug therapy were seen after mitoxantrone treatment but were of no clinical importance. Gastrointestinal toxicity was mild and tolerable. Alopecia was minimal.

Mitoxantrone displayed modest antitumor activity in five of 25 patients, most of whom had been heavily pretreated. Although this activity was of interest in this phase I study, it clearly did not meet the usual criteria of clinical significance.

We would recommend further clinical investigation of a 5-day course in phase II studies of mitoxantrone using a dose of 2.73 mg/m^2 in "good-risk" patients, defined as those who do not have extensive liver metastases and have not been heavily pretreated.

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Phase I Clinical Study With 5′-Deoxy-5-fluorouridine, a New Fluoropyrimidine Derivative[1,2]

Reto Abele,[3,*] Pierre Alberto,[3] Rolando J. Seematter,[3] Ghislaine Germano,[4] Roland Heintz,[4] and Werner Bollag[4,5]

SUMMARY

5′-Deoxy-5-fluorouridine (DFUR) is a new fluoropyrimidine derivative with significant antineoplastic activity in animal systems. Compared to 5-FU or other fluoropyrimidines, DFUR has a more favorable therapeutic ratio in Sarcoma 180-bearing mice. DFUR was studied in this phase I trial with a daily × 5 bolus iv injection. A second course was given ⩾ 3 weeks after the first day of treatment. Doses were escalated from 300 to 5000 mg/m^2/day in 30 patients. Dose-limiting factors were myelosuppression and stomatitis. Hematologic toxic effects were particularly marked on granulocytes. Thrombocytopenia was less frequently encountered. Stomatitis was severe at high doses of DFUR. Eleven patients had nausea or moderate vomiting. Drug-induced myocardial injury may exist, since electrocardiogram changes were recorded in two patients. After rapid iv injection, four patients felt hot in the face and pelvis. Other side effects were minimal. With this daily × 5 schedule of administration, the maximum tolerated dose of DFUR appeared to be 5000 mg/m^2/day. The dose recommended for further clinical use is 4000 mg/m^2/day × 5 for patients previously untreated with chemotherapy.

[Cancer Treat Rep 66:1307–1313, 1982]

5-FU is an antimetabolite extensively used in the treatment of a variety of tumors since 1957 (1). Its toxic effects consist mainly of gastrointestinal intolerance and mucosal lesions, myelosuppression, alopecia, and dermatitis (2). These effects thus limit the amount of drug that may safely be given to patients. Analogs of this class of agents with a possibly higher therapeutic index or improved level of activity have been sought. 5′-Deoxy-5-fluorouridine (DFUR) is a recently synthesized fluoropyrimidine derivative (3). Its chemical structure consists of a 5-FU molecule attached to a pseudopentose. Various other fluoropyrimidine nucleosides, such as 1-(tetrahydrofuryl)-5-fluorouracil (ftorafur) or 2′-deoxy-5-fluorouridine (FUDR), differ in the chemical structure of the sugar moiety (fig 1).

We have chosen to investigate DFUR in a phase I trial, since this compound has shown marked antineoplastic activity against a wide variety of leukemias and solid tumors in animals. Activity has been detected after ip and oral administration (4,5). As compared to other fluoropyrimidines, DFUR possesses a higher therapeutic index in animals. In Sarcoma 180-bearing mice, 50 mg/kg of 5-FU given ip daily for 10 days induces a leukopenia of 1.05 × 10^9 cells/liter. This dose level achieves a tumor growth inhibition of 75% as compared to the tumor growth rate in control animals. In the same model, DFUR induces a leukopenia < 4.0 × 10^9 cells/liter only after doses > 400 mg/kg and inhibits tumor growth to a larger extent. Bollag and Hartmann (5) have further

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[1] Received July 31, 1981; revised Dec 29, 1981; accepted Jan 1982.

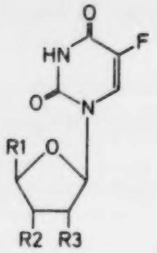
[2] Presented in part at the 72nd annual meeting of the American Association for Cancer Research, Washington, DC, April 1981.

[3] Division of Oncology, University Hospital, Geneva, Switzerland.

[4] F. Hoffmann-La Roche and Co, Ltd, Basel, Switzerland.

[5] We acknowledge the excellent secretarial help of Ms. C. Amez-Droz.

**Reprint requests to:* Dr. Reto Abele, Division of Oncology, University Hospital, 1211 Geneva 4, Switzerland.



compound	R1	R2	R3
1-(tetrahydrofuryl)-5-fluorouracil	H	H	H
2'-deoxy-5-fluorouridine	CH2OH	OH	H
5'-deoxy-5-fluorouridine (DFUR)	CH3	OH	OH

FIGURE 1.—Chemical structure of some fluoropyrimidine nucleosides.

shown that myelosuppressive doses of DFUR are more potent than equitoxic doses of FUDR or ftorafur. Finally, the spectrum of antitumor activity may be different for DFUR than for the other fluoropyrimidines (5).

Animal toxicity studies have been completed in F. Hoffmann-La Roche and Co Laboratories, Basel, Switzerland (6). LD10 for DFUR in normal mice is 543 mg/kg for single iv injection and 360 mg/kg after five daily ip injections. Corresponding doses for rats are > 2000 and 560 mg/kg, respectively. In comparison, the LD10 values for 5-FU are 22 and 24 mg/kg in rats and mice, respectively. In dogs, DFUR and 5-FU induce neurologic symptoms and death after low doses. Gastrointestinal mucosa and endocardium hemorrhages are seen with both compounds.

Transient leukopenia and anemia are observed in rats after prolonged daily administration of DFUR doses > 300 mg/kg. Myelosuppression is also induced by 5-FU, but at daily doses of 10–20 mg/kg. Baboons receiving prolonged daily iv administration do not exhibit leukopenia after DFUR doses up to 80 mg/kg, whereas severe bone marrow toxicity is reported after 5-FU doses of 10 mg/kg. In conclusion, the toxic effects related to DFUR are very similar to those observed after 5-FU administration. However, the doses of DFUR necessary to induce toxic effects are much higher, on a weight basis, than the doses of 5-FU.

In comparison to FUDR, DFUR lacks the hydroxyl group in the 5' position of its pseudopentose. This group appears necessary for phosphorylation and the direct formation of nucleotides (7). This chemical property implies that either DFUR is directly active or that transformation into 5-FU is necessary for its activity. This transformation has been shown to occur with FUDR (7) and ftorafur (8).

The exact mechanisms of action of DFUR are not yet known. DFUR is a substrate for an intracellular nucleoside phosphorylase. Under the action of this enzyme, 5-FU and other phosphorylated metabolites have been rapidly detected in Ehrlich ascites tumor cells (9). Phosphorylase activity is particularly high in Sarcoma 180 tumor cells as compared to normal mouse cells, with the exception of small intestinal tissue (6). Phosphorylase activity variation among different cell types and/or particular metabolic activation may explain the better tolerance of normal tissue to DFUR as compared to other fluoropyrimidines. This seems of special importance for the bone marrow, explaining the higher myelotoxicity of 5-FU compared to the same dose of DFUR.

MATERIALS AND METHODS

Patients with advanced malignancies were eligible for the trial if they had a leukocyte count $> 3.5 \times 10^9$/liter, a platelet count $> 120 \times 10^9$/liter, a serum bilirubin < 20 µmols/liter, and a serum creatinine < 120 µmols/liter. Performance index had to be > 40 on the Karnofsky scale. Further, expected followup compliance and patient informed consent were required.

DFUR was supplied by F. Hoffmann-La Roche and Co, Ltd, Basel, Switzerland, in vials containing 1000 mg of pure substance as a 10% sodium salt. Immediately prior to use, vials were dissolved with 10 ml of sterile water for injection. DFUR was given daily for 5 days by a bolus iv injection of the solution (100 mg/ml). A second course was administered on Day 22 or later after recovery from toxicity. This daily × 5 schedule was chosen because it is widely used for 5-FU and thus allows a comparison of clinical tolerance between fluoropyrimidine analogs.

The starting dose of DFUR was 300 mg/m^2/day, approximately one third of the mouse LD10 (10). An original scheme was used to rapidly escalate the DFUR doses after the first step (table 1). We anticipated from animal data that the maximum tolerated dose in man should be ten to 15 times higher than the corresponding dose for 5-FU. No intrapatient dose escalation was allowed in this trial. Patients were carefully monitored for signs of clinical toxic effects at least once a week. A complete hemogram was scheduled twice a week during the treatment period, with a blood chemistry weekly. The lowest hematologic counts were separately reported for each patient in the first and subsequent courses of treatment with DFUR. An ECG and audiogram were required before and after completion of the trial. Tumor response was determined after at least two courses with DFUR in patients with measurable or evaluable lesions, using standard criteria of response (11).

The pharmacokinetics of the unchanged drug was also studied. Ten blood samples were drawn into potassium oxalate-containing vacuum tubes at selected times during the first 4 hours after the DFUR bolus injection of Day 1. Samples were also taken after 24 and 96 hours. Test tubes were placed on ice and centrifuged within 1 hour at 4°C. Plasma was then transferred into glass tubes, immediately frozen at -20°C, and stored until drug measurement. At this time, 1 ml of plasma was diluted with an equal volume of saturated ammonium sulfate solution and extracted twice with six volumes of ethyl acetate. The organic phases were then combined and evaporated. The dry extract was later diluted in 1 ml of a mixture of water/methanol/acetic acid (94/6/0.1 vol/vol). An equivalent 80 µl of plasma was subjected to high-performance liquid chromatography separation on reverse phase RP18 with water/methanol/acetic acid (94/6/0.1 vol/vol) as eluant. The drug was quantified at 268 nm using an ultraviolet detector. The sensitivity limit of the method was 50 µg/ml of plasma.

TABLE 1.—Escalation scheme for DFUR

Starting dose	DFUR dose (mg/m^2/day)	Relative increase above preceding dose level (%)
1 n	300	
3.3 n	1000	233
6.6 n	2000	100
10.0 n	3000	50
13.3 n	4000	33
16.6 n	5000	25

RESULTS

Thirty patients were treated with DFUR. Patient characteristics are shown in table 2. Most of the patients were previously untreated: only four of them had prior chemotherapy, including two who received 5-FU. One patient had been treated with radiation therapy.

Sixty-six 5-day courses of DFUR were administered. Eight patients had only one course of DFUR. No patient received more than four courses. Except at the highest dose level of 5000 mg/m^2/day, four to eight patients were entered at each dose level.

Tables 3–6 show the hematologic toxicity induced by DFUR administration. After the first course of treatment, no leukocyte reduction was observed in the first three dose levels, up to 2000 mg/m^2/day. At the next step, two patients showed a moderate leukopenia of 2.1 and 3.1 × 10^9 cells/liter. Four of seven patients treated with 4000 mg/m^2/day had a leukopenia $< 4.0 \times 10^9$ cells/liter, with two of them having a count $< 1.0 \times 10^9$ cells/liter. At this dose level, the median time to nadir was 18 days, with a range of 9–22 days. Duration of leukopenia ranged from 2 to 6 days after nadir.

The two patients treated with 5000 mg/m^2/day had lowest leukocyte counts of 0.6 and 2.4 × 10^9/liter. Granulocytopenia was particularly severe. One of these patients had a gram-negative sepsis, and both patients were treated with broad-spectrum antibiotics while granulocytopenic. Recovery, as determined by leukocyte counts $> 4.0 \times 10^9$/liter, occurred on Days 19 and 27 at this dose level.

Up to 4000 mg/m^2/day, leukocyte toxicity induced in subsequent courses with DFUR was not more

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Patients in study	30
Female/male	15/15
Median age in yrs (range): 63 (38–82)	
Median performance index (range): 70 (50–80)	
Primary tumor	
Lung, non-small cell types	6
GI tract adenocarcinoma	20
Other	4
No prior treatment	25
Radiotherapy alone	1
Chemotherapy alone	4

TABLE 3.—DFUR-induced leukocyte toxicity after 1st course of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Median lowest count (range), × 10⁹ cells/liter		Median No. of days to nadir (range)
		Leukocytes	Granulocytes	
300	2/5	9.7 (8.8–10.6)	8.3 (7.5–9.1)	8 (6–9)
1000	7/8	7.3 (4.0–11.6)	4.5 (2.8–9.2)	9 (5–22)
2000	4/4	6.2 (3.9–11.0)	3.5 (2.5–4.4)	15 (5–19)
3000	4/4	4.1 (2.1–6.3)	2.2 (0.7–3.9)	22 (18–22)
4000	6/7	2.3 (0.6–5.3)	0.8 (0.1–2.7)	18 (9–22)
5000	2/2	1.5 (0.6–2.4)	0.02 (0.0–0.03)	17 (17)

TABLE 4.—DFUR-induced leukocyte toxicity after subsequent courses of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Median lowest count (range), × 10⁹ cells/liter		Median No. of days to nadir (range)
		Leukocytes	Granulocytes	
300	2/5	11.9 (11.3–12.4)	NA*	4 (4)
1000	4/5	5.4 (3.2–6.7)	3.5 (1.7–5.2)	8 (5–19)
2000	4/4	6.1 (3.8–9.2)	3.1 (2.6–5.3)	12 (7–25)
3000	4/4	5.1 (4.1–6.7)	3.8 (2.1–4.5)	20 (5–22)
4000	2/2	4.5 (4.4–4.5)	1.5 (1.3–1.7)	20 (18–22)

*Not available.

severe than in the first course, and the patients could be re-treated with the same dose of DFUR (table 4). The two patients receiving 5000 mg/m²/day were re-treated with 3000 mg/m²/day.

Thrombocytopenia < 100 × 10⁹ cells/liter was seen in several patients after the first or subsequent course with DFUR (tables 5 and 6). Platelet toxicity was encountered at low dose levels after the first or second course with DFUR in patients with a poor performance index or renal insufficiency. Thrombocytopenia was recorded in two of seven patients given 4000 mg/m²/day and in one of two patients treated with 5000 mg/m²/day. Platelet recovery to > 100 × 10⁹/liter was rapid in every patient, except one who received 300 mg/m² daily × 5 and died on Day 41 from a gastrointestinal hemorrhage while thrombocytopenic. Autopsy revealed ischemic hemorrhagic necrosis of the jejunum.

Nonhematologic toxic reactions induced by DFUR administration are shown in table 7. Gastrointestinal tolerance was good in most patients. However, 11 patients experienced anorexia, nausea, or discrete vomiting.

Stomatitis was observed in six patients, was severe in four of them, and was seen at various dose levels. After 1000 mg/m²/day, two patients developed stomatitis: mucosal ulcerations were observed in one, an 82-year-old patient treated in error, while having a threefold increase of serum creatinine. The two patients receiving 5000 mg/m²/day developed severe stomatitis with ulcerations. They had no known risk factors.

Precordial pain, appearing during the days of DFUR administration, was observed in two patients treated with 1000 mg/m²/day. ECG changes consisting of ST segment displacements were recorded, and one patient had a significant transient cardiac enzyme elevation. No similar toxic reaction was observed after higher doses of DFUR.

Neurologic symptoms were observed in three patients. Transient tremor, ataxia, and somnolence were seen after 300 and 3000 mg/m²/day of DFUR,

TABLE 5.—DFUR-induced platelet toxicity after 1st course of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Lowest count, × 10^9 cells/liter		No. of days to nadir	
		Median	Range	Median	Range
300	2/5	389	173–605	8	6–9
1000	7/8	234	62–319	14	5–19
2000	4/4	217	164–380	15	5–21
3000	4/4	185	167–200	15	9–18
4000	5/7	120	30–204	12	9–19
5000	2/2	108	29–186	16	15–17

TABLE 6.—DFUR-induced platelet toxicity after subsequent courses of treatment

Dose (mg/m²/day)	No. of patients evaluable/total No. of patients	Lowest count, × 10^9 cells/liter		No. of days to nadir	
		Median	Range	Median	Range
300	2/5	133	58–207	12	10–14
1000	4/5	195	88–208	12	5–19
2000	4/4	196	157–312	14	10–21
3000	4/4	162	105–260	6	2–9
4000	2/2	134	131–137	9	8–10

TABLE 7.—DFUR-induced nonhematologic toxic reactions

	No. of toxic reactions at each dose of DFUR (mg/m²/day)					
Type of toxic reaction	300	1000	2000	3000	4000	5000
Nausea and vomiting	1	1	3	2	3	1
Stomatitis	0	2	1	0	1	2
ECG changes	0	2	0	0	0	0
CNS symptoms	2	0	0	1	0	0
Sensation of heat	0	0	1	0	2	1
Alopecia	0	0	0	0	0	1
No. of patients with toxic reactions/ total No. of patients	3/5	4/8	4/4	3/4	5/7	2/2

respectively. Audiogram changes with loss of high frequency detection were observed in one of only four evaluable patients.

Four patients reported a sensation of heat in the face and pelvis. This phenomenon appeared only after very rapid iv injection of at least 2000 mg/m²/day.

Finally, complete alopecia was seen in one patient treated with 5000 mg/m²/day. No vein induration or skin discoloration secondary to treatment was seen.

A total of 12 patients were evaluable for response to DFUR. Antitumor effect was observed in two previously untreated patients who received 2000 mg/m²/day of DFUR. The first patient had < 50% regression of hepatic metastases of a gastric adenocarcinoma, as measured by liver ultrasonography; the second had an almost complete disappearance of a pleural effusion from an adenocarcinoma of the lung, without drainage of the pleural cavity. Tumor stabilization was seen in seven patients after documented disease progression. One of these patients with a colon adenocarcinoma had responded previously to 5-FU.

Seven patients died before completion of two courses of DFUR. Two deaths were related to infectious complications secondary to granulocytopenia in patients treated with 4000 mg/m²/day. As mentioned above, one patient died from massive gastrointestinal bleeding. All of the other deaths were attributable to tumor progression.

In summary, DFUR doses in the range of 4000–5000 mg/m²/day led to severe hematologic and nonhematologic side effects. We felt that further treatment with 5000 mg/m²/day was not warranted. Dose-limiting factors were clearly myelosuppression and stomatitis. We thus considered that the dose of 5000

TABLE 8.—Pharmacokinetic parameters after DFUR iv bolus injection

	Dose (mg/m²/day)				
Parameter	600 (2 patients)	1000 (3 patients)	2000 (3 patients)	3000 (2 patients)	4000 (2 patients)
Mean terminal half-life (mins)	20.5	16.5	25.8	23.4	22.8
Mean distribution volume (liters)	30	18	21.3	26	17
Mean area under the curve (μg/ml × hr)	10.2	30	85.9	112	220
Mean clearance (liters/hr)	127	83	39	42	30

mg/m²/day was the maximum tolerated dose for man when using a daily × 5 schedule for administration of DFUR.

The pharmacokinetics of the unchanged drug has been studied in 12 patients during the first day of DFUR administration. Dose levels have ranged from 600 to 4000 mg/m²/day. Pharmacokinetic data for the unchanged DFUR are the best fit based on a two-compartment open model. The beta half-life values for doses up to 4000 mg/m²/day range from 16.5 to 25.8 minutes (mean, 21.8 ± 3.5) (table 8). This short half-life is independent of the dose of DFUR. On the other hand, the area under the curve increases unproportionally with the dose. As a consequence, the total body clearance does not remain constant but diminishes with escalating doses.

DISCUSSION

Several fluoropyrimidine analogs have been proposed in recent years in the search for a drug with a better therapeutic index and an increased level or broader spectrum of activity in comparison to 5-FU. Overall results have not shown significant advantages of any of these derivatives. Ftorafur does not yield improved activity over 5-FU and has severe toxic effects. Central nervous system side effects, which are dose-limiting, have been correlated with sustained cerebrospinal fluid drug levels (12).

The results of our phase I trial with DFUR show that myelosuppression is induced by this new fluoropyrimidine derivative at doses > 2000 mg/m²/day × 5. This compound appears to be more toxic for leukocytes than for platelets at the dose levels tested. Within a narrow range of dose increments from 3000 to 5000 mg/m²/day, a leukopenia < 4.0 × 10^9 cells/liter is observed in most patients. Granulocytes are particularly affected. Treatment with DFUR is thus limited by a dose-related leukopenia. Our results do not show evidence for a cumulative myelosuppression after repeated courses with DFUR.

On the other hand, nonhematologic effects seem acceptable. Gastrointestinal tolerance has been good in most patients. However, stomatitis has been seen and is a dose-limiting factor in DFUR treatment. Sensation of heat in the face and pelvis has been observed after rapid iv administration. Similar symptoms have been reported for another 5-FU derivative, 1-hexylcarbamoyl-5-fluorouracil, after oral administration (13). Further cardiac evaluation should be done since DFUR may induce myocardial damage, as evidenced by ECG changes in two of our patients. Cardiac toxicity has also been reported for 5-FU (14,15). Finally, alopecia has been observed in our patients. In summary, DFUR induces nonhematologic side effects closely related to those reported after 5-FU administration. However, the doses necessary to induce these toxic effects are much higher than those of 5-FU.

In conclusion, DFUR can be given at higher doses than 5-FU with an acceptable tolerance. Its toxicity seems to be strongly dose-related, since small dose modifications may sharply increase the severity of the drug-induced toxic reactions. In the search for new active antitumor compounds, DFUR warrants further clinical trial. We propose a dose of 4000 mg/m²/day × 5 every 3–4 weeks for phase II trials in patients untreated previously with chemotherapy. We think that the antineoplastic activity of DFUR has to be established first in tumor types usually sensitive to 5-FU, such as gastrointestinal tumors. The Swiss Group for Clinical Cancer Research (SAKK) has initiated a phase II trial with DFUR in advanced colorectal adenocarcinomas. The activity of DFUR in other tumor types should also be assessed later.

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ctivity of Isotretinoin Against Squamous Cell Cancers and reneoplastic Lesions[1,2]

ank L. Meyskens, Jr,* Eileen Gilmartin, David S. Alberts, Norman S. Levine, Robert Brooks, ydney E. Salmon, and Earl A. Surwit[3,4]

UMMARY

We investigated the effect of the synthetic vitamin A derivative isotretinoin (13-*cis*-retinoic acid) on advanced cancers in 103 patients and on preneoplastic lesions in five patients. Six of 14 patients with squamous cell epithelial cancers had objective regressions of skin or subcutaneous metastases. Three of five patients with preneoplastic lesions had objective responses. The major dose-limiting toxic effects were reversible dermatitis, emotional lability, and headaches. We conclude that the growth of some squamous cell epithelial malignancies can be inhibited by isotretinoin and suggest that other retinoids should be evaluated as antitumor agents.

[Cancer Treat Rep 66:1315–1319, 1982]

Vitamin A is necessary for the normal development of all epithelial tissues (1). This vitamin and its natural and synthetic derivatives (retinoids) have shown significant activity as chemopreventive agents in numerous animal systems, whether the tumor was initiated by chemical or by physical carcinogens (2,3). The retinoids have also shown antiproliferative activity against a large number of animal and human malignant cell lines (4–9). The growth-suppressive effect against cell lines from melanoma (7–9), teratocarcinoma (10), and promyelocytic leukemia (11) has been accompanied by differentiation. In tests on fresh biopsy material from human tumors, we have also observed that retinoids can sometimes inhibit tumor colony formation in soft agar (12,13). Furthermore, several of the retinoids, including the synthetic vitamin A derivative isotretinoin (13-*cis*-retinoic acid), have shown antiproliferative activity against such dermatologic conditions as psoriasis, rare epithelial dysplasias, and basal cell carcinomas (14,15). No serious cytotoxic effects were recorded in these investigations or in the treatment of a large number of patients with conglobate acne (16).

These results suggest that in addition to chemopreventive effects retinoids may have antineoplastic activity. We have therefore conducted a broad phase II trial of isotretinoin to assess its antineoplastic activity and have detected activity in preneoplastic and neoplastic lesions of squamous cell histology.

MATERIALS AND METHODS

Patient characteristics.—Patients were eligible for treatment with isotretinoin if they had advanced cancer or aggressive preneoplastic conditions with measurable disease that could be evaluated for regression. Patients with advanced cancer received isotretinoin at a dose of 3 mg/kg/day orally, while patients with preneoplastic lesions were started at a dose of 2 mg/kg. These doses were empirically selected based on the extensive experience with iso-

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[1] Received Oct 2, 1981; revised Jan 18, 1982; accepted Jan 27, 1982.

[2] Supported in part by Public Health Service grant CA-17502 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Hoffmann-La Roche, Inc.

[3] Departments of Internal Medicine and Obstetrics and Gynecology, and the Cancer Center Division, The University of Arizona, Tucson.

[4] We thank W. Scott of Hoffmann-La Roche for supplying isotretinoin; B. Pawson for her interest in our work; the physicians who referred patients for this investigation, particularly B. Durie, D. Franks, R. Jackson, S. Jones, S. Ketchel, T. Miller, and A. Wendt; and R. Markmann for excellent secretarial assistance.

**Reprint requests to:* F. L. Meyskens, Jr, MD, Department of Internal Medicine, Section of Hematology and Oncology, 6th Floor Facility, The University of Arizona, Health Sciences Center, Tucson, AZ 85724.

TABLE 1.—Patient distribution and response rate of eligible and evaluable patients treated with isotretinoin

		No. (%) of responses*	
Histologic class	No. of patients	Including mixed responses	Excluding mixed responses
Epithelial†			
Squamous cell			
Advanced	24	6 (25)	2 (8)
Low-grade (preneoplastic)	5	3 (60)	3 (60)
Non-squamous cell	45	1 (2)	1 (2)
Malignant melanoma	13	1 (7)	1 (7)
Nonepithelial†	21	0	0

*Responses include only mixed, partial, and complete; improvements are not included.

†Tumor types included: epithelial squamous cell, high-grade (8 lung, 10 head and neck, and 6 other); epithelial squamous cell, low-grade (3 multiple basal cell, 1 keratoacanthoma, and 1 epidermal dysplasia verruciformis); epithelial non-squamous cell (8 breast, 12 ovarian, 12 colon, 4 bladder, and 9 miscellaneous); and nonepithelial (3 choriocarcinoma, 5 lymphoma, 4 leukemia, and 9 miscellaneous).

tretinoin against dermatologic lesions (14–16). In a pilot study, doses were escalated by 0.5-mg/kg increments every 1–2 weeks to limiting toxicity in the first 16 patients with advanced cancer. It became clear that most patients (12 of 16) would not tolerate > 4 mg/kg of isotretinoin for much longer than 3–4 weeks. Therefore, subsequent patients were started and maintained at a dose of 3 mg/kg of retinoid, and the toxic effects cited represent side effects at this level of drug. To be eligible for evaluation, patients could not have received chemotherapy within 4 weeks or radiation therapy within 16 weeks of entering the study, and they had to receive daily therapy with isotretinoin for a minimum of 1 month.

Response criteria.—Response criteria were the following: *complete response*—the disappearance of all measurable disease; *partial response*—a > 50% decrease of the sum of the products of the perpendicular diameters of all measurable lesions; *mixed response*—a > 50% decrease in a measurable disease site while other sites of disease showed no regression; *improvement (minor response)*—a > 25% but < 50% decrease in total measurable disease; and *progression*—a > 25% increase of any disease parameter.

Evaluation for toxicity.—Patients were serially monitored. A careful physical examination was done every 2 weeks, and liver function studies (LDH, SGOT, SGPT, bilirubin), cbc, and prothrombin time were done monthly. These studies were also repeated when the patient discontinued therapy. A radionuclide liver/spleen scan was obtained in all patients who had been receiving therapy for at least 4 months and was repeated every 4 months thereafter.

RESULTS

A total of 143 patients were entered in this investigation. Thirty-five patients were considered ineligible or inevaluable for the following reasons: no objectively evaluable disease (18 patients), early death (nine), discontinuation of isotretinoin after 2–3 weeks secondary to "rapid progression" (five), and intolerable cutaneous side effects (three).

Results are reported for 108 eligible and evaluable patients. The patient distribution and responses were grouped by histologic class (table 1). Considerable activity of isotretinoin was seen in patients with squamous cell epithelial disease (table 1). The six objective responses in cancer patients and the responses of the three patients with preneoplastic lesions are detailed in table 2. All responses occurred in subcutaneous or skin disease sites. In 45 patients with non-squamous cell epithelial malignancies, one partial response was seen in a patient with ovarian cancer which included a > 50% decrease in a large pelvic mass and complete disappearance of refractory ascites. This response lasted for 2 months, when the patient discontinued therapy unexpectedly on her own volition. Two minor responses (1-month duration) of skin disease from breast cancer were also seen.

Thirteen patients with metastatic malignant melanoma were treated, and one patient had a > 50% decrease in a large (13 × 10-cm) subcutaneous mass

TABLE 2.—Details of responses of patients with neoplastic or preneoplastic squamous cell disorders to isotretinoin

Patient No.	Diagnosis	Prior treatment	Response description	Response	Response duration (mos)
			Neoplastic Disorders		
7	Squamous cell, nasopharynx	5 drugs + radiation therapy	50% decrease in 8 × 5-cm neck mass	Partial	2
133	Squamous cell, epiglottis	Methotrexate	75% decrease in massive (> 12 × 10-cm) neck and jaw mass; resolution of periorbital edema	Partial	2
[illegible]	Squamous cell, lung	Radiation therapy	50% decrease in 7 × 9-cm jaw mass; bone lesions stable	Mixed	2
145	Squamous cell, lung	Radiation therapy	Disappearance of axillary lesion (2 × 3-cm); no change in opacified chest roentgenogram	Mixed	7
80	Squamous cell, ear	Radiation therapy	Disappearance of 3 finger lesions and 1 arm lesion; other lesions stable	Mixed	1
3	Squamous cell, face	Multiple surgery, radiation therapy	Remodeling of face; stable orbital lesion	Mixed	10
			Preneoplastic Disorders		
109	Keratoacanthomas	Multiple surgery	Disappearance of 24 of 25 lesions, resected (0.5–2-cm)	Partial	12+
43	Multiple basal carcinomas	Concurrent radiation to 2 large face lesions	Complete response of several unirradiated areas; partial response of additional areas	Partial	16+
154	Epidermal dysplasia verruciformis	None	Resolution of 50% of total mass of multiple lesions	Partial	2*

*Discontinued due to skin toxicity; prompt relapse followed.

for 4 months. No responses were seen in 21 patients with nonepithelial malignancies. The pretreatment characteristics of the patients who responded to isotretinoin did not appear to vary significantly from those of patients who did not respond. Careful stratification and more extensive studies of patients with squamous cell malignancies and malignant melanoma are needed and will be required to substantiate this conclusion.

The side effects of isotretinoin at 3 mg/kg are shown in table 3. No bone marrow or hepatic toxicity was recorded. Nausea and vomiting were very mild. Two patients discontinued therapy secondary to skin toxicity after 6 weeks of therapy, and four patients discontinued therapy secondary to emotional lability or headaches after 6, 8, 12, and 16 weeks of therapy. Twenty-three patients also had serial lipid value measurements. Increases in plasma triglyceride levels were recorded in 12 patients. Isotretinoin was not discontinued, and in six patients who lived long enough, additional serum lipid values were obtained 1 month after completing therapy. Plasma triglyceride levels had returned to normal.

DISCUSSION

In the past decade, therapeutic activity of isotretinoin has been reported in numerous dermatologic conditions (15–17). The present study suggests that isotretinoin has antitumor activity in advanced squamous cell epithelial cancers. Our results also confirm that isotretinoin has antiproliferative activity in preneoplastic lesions such as keratoacanthomas (18) and basal cell carcinoma (15). Bollag (19) had noted previously that epithelial tumors of the skin were affected by retinoids. The responses documented here demonstrate that isotretinoin can also inhibit neoplastic squamous cell epithelial lesions in cutaneous and subcutaneous sites. The site selectivity of many of the responses is of interest and warrants further investigation with regard to mechanism.

The biologic actions of retinoids are probably mediated both indirectly by host cells (20,21) and by a number of mechanisms (22). Data suggest that a major effect is modulated through an intracellular binding protein. This receptor molecule has been

TABLE 3.—Toxicity of isotretinoin in phase II trial

Type of effect	Patient reaction
Bone marrow	None
Hepatic	None
Joint pain	Mild (10%–15%)
Nausea and vomiting	6 patients (mild); 2 patients discontinued
Skin and mucosa*	Mild (95%); 2 patients discontinued, 6 patients lowered dose
CNS	Mild emotional changes or headaches (25%); 4 patients discontinued
Lipid	25% increase in triglyceride level in 12 of 23 patients followed serially

*Cheilitis, dermatitis, conjunctivitis.

identified in many human tumors (23–26); however, in some experimental systems the antiproliferative effect of retinoic acid has not always been associated with the presence of retinoic acid-binding protein (6).

The proportion of ineligible and inevaluable patients was high (24%) compared to our usual rate of 5%–7% in phase II investigations. The large number of patients without objectively evaluable lesions (12.6%) or with early death (6.3%) reflected the attitude of "it's only a vitamin and can't harm the patient," even among a staff that was relatively sophisticated about retinoids. Three percent of the patients appeared to have increased progression of their disease, an incidence that is similar to other phase II studies and probably represents the natural history of the underlying disease process. Nevertheless, isotretinoin may in part function as a hormone, and careful observation for this phenomenon should be made in phase III studies.

The toxicity of isotretinoin was different qualitatively and quantitatively from that of most cytotoxic anticancer drugs. The predominant limiting side effects occurred in skin. Additionally, mild CNS changes consisting of emotional lability and headaches were frequent; this finding was anticipated, as similar changes with retinyl palmitate have previously been reported (27). However, these symptoms are different than those seen in patients with dermatologic conditions, and the presence of headaches and emotional lability probably reflects the higher dose used in our studies. Perhaps of relevance is that retinoids are known to concentrate in the brain (28). In contrast to vitamin A, isotretinoin produced no abnormalities in liver function, a finding consistent with the lack of retention of isotretinoin by the liver (28). The increase in triglycerides, which was seen in the 12 of 23 patients in whom this lipid was measured, is similar to results obtained in patients with dermatologic conditions (29). This effect appears to be dose-dependent, is rapidly reversible upon retinoid discontinuation, and does not involve lipid subclasses correlated with atherosclerotic disease. Nevertheless, cancer patients who will receive retinoids long-term should be carefully monitored for lipid abnormalities.

Many retinoids have been synthesized (4). In dermatologic conditions, isotretinoin and an aromatic derivative (etretinate) have a totally different spectrum of activity (19). We used isotretinoin in this trial because its toxicity had already been studied and found to be acceptable in noncancerous conditions, and an oral formulation was available. Our results in squamous cell neoplasms suggest that other retinoids should also be evaluated for antitumor activity (30,31). Additionally, the evidence of isotretinoin activity that we observed in squamous cell epithelial malignancies provides support for the use of retinoids in additional studies of some of these tumor types as well as in chemoprevention studies.

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Clinical and Clinical Pharmacologic Studies of Aziridinylbenzoquinone[1,2,3]

John P. Griffin, Robert A. Newman, John J. McCormack, and Irwin H. Krakoff[4,5,*]

SUMMARY

Thirty patients were entered in a phase I trial of aziridinylbenzoquinone (AZQ). These patients were treated with a single iv dose of AZQ daily for 5 days at doses ranging from 0.5 mg/m² to 10.0 mg/m². The dose-limiting and only significant toxic effects were leukopenia and thrombocytopenia, which appeared with relative consistency at iv dosages of 4.0–4.5 mg/m²/day × 5 in patients with extensive prior treatment and 7.0 mg/m²/day × 5 in patients with little prior treatment. One patient with colon cancer achieved a partial response of 4+ months. No other evidence of antitumor activity was observed. Pharmacologic studies revealed that the drug disappeared from the plasma in a biphasic manner (α-half-life = 1.5 minutes, β-half-life = 18 minutes). With our method of analysis by high-performance liquid chromatography, no metabolites were found in the serum.

[Cancer Treat Rep 66:1321–1325, 1982]

Aziridinylbenzoquinone (AZQ) is of interest for a phase I clinical trial for two major reasons. (*a*) AZQ has significant activity against a number of transplantable murine tumors including L1210 lymphoid leukemia, P388 leukemia, Colon 26 tumor, and CD8F mammary tumor (1,2); and (*b*) AZQ possesses a chemical structure in which the properties of lipid solubility, low ionization, and water solubility are optimized (1–4). Both lipid solubility and low ionization are believed to be important in the penetration of drugs into the central nervous system (5). Not only does AZQ penetrate and attain significant concentration in the central nervous system of animals (6), but it also has shown significant antitumor activity against intracranially implanted L1210 and P388 leukemias and an ependymoblastoma when given ip (1,2).

At the present time the exact mechanism of action of AZQ is unknown, although it is thought to possess alkylating activity against DNA (1,7). An additional mechanism of activity of this drug may involve free-radical formation, which could account for some of its antitumor activity (8). Preclinical toxicologic studies in mice, dogs, and monkeys (1) indicated that the major toxicity of AZQ was in the gastrointestinal, hematopoietic, and lymphatic systems; lesser toxicity was noted in the respiratory, hepatic, nervous, and renal systems. The schedule of treatments appeared to have little effect on the toxicity. With a 5-day schedule, the toxic dose low for the most sensitive species studied (beagle dog) was 1.6 mg/m². The present phase I study was undertaken to determine the characteristics of toxicity, the minimum dose that produces consistent reproducible evidence of biologic effect, and the maximally tolerated dose of AZQ in humans, when given iv daily for a 5-day course.

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[1] Received Apr 20, 1981; revised Dec 16, 1981; accepted Jan 5, 1982.

[2] Supported by contract N01-CM-97278 (Division of Cancer Treatment) and Public Health Service grant CA-24543 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Presented in part at the April 1981 meeting of the American Association for Cancer Research.

[4] Vermont Regional Cancer Center and the Departments of Medicine (*J. P. Griffin* and *I. H. Krakoff*) and Pharmacology (*R. A. Newman*, *J. J. McCormack*, and *I. H. Krakoff*), University of Vermont, Burlington.

[5] We are grateful for the skillful assistance of Cynthia Ackerley in HPLC analysis and Martha Dewees in clinical data management.

*_Reprint requests to:_ Irwin H. Krakoff, Vermont Regional Cancer Center, University of Vermont, 1 S Prospect St, Burlington, VT 05401.

MATERIALS AND METHODS

All patients selected for this study had advanced, histologically proven cancer for which conventional therapy had been exhausted or for which there was no conventional or investigational therapy with a reasonable expectation of benefit. AZQ was administered in the wards and clinics of the Medical Center Hospital of Vermont. The nature of the study was explained to each patient, who signed an appropriate informed consent before the study began. Patients who entered the study had a life expectancy of at least 8 weeks, had a Karnofsky performance status ≥ 30, and were > 18 years of age.

AZQ was given iv. Each 10-mg vial was reconstituted with 0.5 ml of sterile *N,N*-dimethylacetamide and 9.5 ml of sterile 0.01 M phosphate buffer (pH 6.5), to a final concentration of 1 mg/ml. The daily dose was further diluted to 20 ml with normal saline and given by iv push over a period of 30–60 seconds. The daily dose was repeated for 5 days, and each course was repeated no more frequently than every 3 weeks. All patients were treated until toxic effects were observed. If there was evidence of therapeutic response, the drug was continued until disease progression was demonstrated. The starting dose was 0.5 mg/m²/day × 5 (⅓ the toxic dose low in the dog) and dose escalation followed a modified Fibonacci scheme. Baseline data included a history, physical examination, and review of the microscopic slides used to make the diagnosis. Initial laboratory studies included a cbc and platelet count, bone marrow examination, urinalysis, biochemistry screening profile,[6] and other studies determined to be necessary on the basis of tumor type and the clinical condition of the patient. Followup studies included discussion of symptoms, physical examination, and cbc, daily while receiving AZQ and twice weekly thereafter. Biochemistry screening and urinalysis were done weekly during and for 3 weeks following AZQ administration. X-rays, scans, etc, were done as necessary to evaluate changes in organ function that might be due to growth or regression of tumor or due to drug toxicity.

The concentration of AZQ in the serum and cerebrospinal fluid was determined by a high-performance liquid chromatography (HPLC) method developed in our laboratories, as has been previously described (9) and is summarized here.

A Spectra Physics model 8000 microprocessor-controlled high-performance liquid chromatograph equipped with a data system was used. The chromatograph was equipped with a Schoeffel model 770 variable-wavelength ultraviolet detector set at 340 nm. The column was an Altex (Berkeley, CA) Ultrasphere IP 5-μm column (25 cm × 4.6 mm ID). A guard column (7 cm × 0.2 cm ID) packed with Co:Pell ODS (25–37 μm; Whatman, Clifton, NJ) was installed to protect the 5-μm column. Samples were injected onto the column through a 10-μl loop, using a manual injector. For each time point studied, 10 ml of whole blood was obtained from patients by venipuncture in the arm opposite that used for injection of AZQ. Serum was obtained by centrifugation and stored at –10°C for < 48 hours prior to analysis.

Serum samples were prepared for injection onto the column using Sep-Pak cartridges (Waters Assoc, Milford, MA). The use of the Sep-Paks affords both sample purification and drug concentration. Cartridges were prepared for use as described in the brochure supplied by Waters Assoc. A measured volume (0.5–4.0 ml) of serum or stock drug solution was loaded onto the Sep-Pak, which was then washed with 1.0 ml of HPLC-grade water containing PIC reagent A. AZQ was then eluted by washing the Sep-Pak with 3.0 ml of HPLC-grade methanol containing 5 mM tetrabutylammonium phosphate (PIC reagent A: Waters Assoc). The methanol was removed under nitrogen, and the residue was taken up in a minimum volume (100–200 μl) of HPLC-grade water. The sample was vortexed and then centrifuged to remove the insoluble material. The resulting solution was used directly for injection onto the column.

AZQ was eluted from the column using an isocratic mobile phase of water:methanol (65:35, vol/vol) containing 0.005 M tetrabutylammonium phosphate (PIC reagent A). A flow rate of 1.0 ml/minute was used. The concentration of AZQ in the eluate was measured by ultraviolet detection at 340 nm. Quantitative analysis was based on peak areas and was computed using preset integration programs in the data system of the Spectra Physics HPLC instrument. Prior to sample analysis, the chromatograph was calibrated daily using AZQ solutions of known concentration.

The minimum detectable level of AZQ was 7.0 ng injected, at a detector setting of 0.01 aufs and a recorder attenuation of 0. The signal-to-noise ratio was ≤ 6 under these conditions.

To determine the recovery of AZQ from plasma, samples of human plasma were spiked with known concentrations of AZQ and then extracted using the Sep-Pak cartridges. Even when the plasma samples were spiked with relatively low concentrations of AZQ (eg, 1–3 μg/ml), the recovery of the drug from the plasma was ≥ 90% as calculated by the data system of the chromatograph. Recovery of AZQ from human serum samples was identical to that obtained in human plasma samples. Blood samples were obtained 1, 2, 5, 10, 20, 30, and 60 minutes after injection of AZQ. Serum AZQ half-times were calculated directly from the data, using least squares regression linear analysis.

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[6] Serum chlorides, CO_2, potassium, sodium, urea nitrogen, creatinine, uric acid, glucose, alkaline phosphatase, total and direct bilirubin, SGPT, SGOT, lactic dehydrogenase, total protein, albumin, calcium, phosphorus, and cholesterol.

RESULTS

Clinical Studies

Thirty patients (table 1) were enrolled in the study (23 evaluable). A total of 46 courses of AZQ were given (37 were evaluable; eight courses were inevaluable due to early death from disease before toxicity could have occurred). The major dose-limiting toxic effect was bone marrow depression (table 2). In patients with extensive prior chemotherapy and/or radiotherapy (> 6 months of prior chemotherapy, > 2 months of chemotherapy and/or large-field radiotherapy, or five or more radiation therapy fields), this toxic effect occurred relatively consistently at a dose of 4.0–4.5 mg/m²/day × 5, while in patients with little prior treatment, it did not occur with relative consistency until a dose of 7.0 mg/m²/day × 5 was reached. All patients treated at a dose of 10 mg/m²/day × 5 developed severe leukopenia or thrombocytopenia; the median wbc count nadir occurred on Day 21, and the median platelet count nadir occurred on Day 20. In this study, there were no drug-related renal, hepatic, respiratory, mucosal, central nervous system, or cardiac toxic effects, and there was no alopecia or nausea and vomiting. Sixteen of the 46 courses were preceded by one to three prior courses at the same or lower doses. These revealed no definite evidence of cumulative toxicity. Since no patients had abnormal renal function and only one patient had a transient increase in bilirubin, we were unable to draw any conclusions about the effect of these variables on AZQ toxicity. The tumor types treated in this study are listed in table 1. One patient with colon cancer achieved a partial response for 4+ months (a > 50% decrease in the product of two perpendicular diameters of a palpable rectal mass and a > 30% decrease in the total size of the liver in the midclavicular line). None of the other patients showed evidence of response.

TABLE 1.—Characteristics of patients given AZQ

Characteristic	No. of patients
Age in yrs	
Median: 59.5	
Range: 23–78	
Sex	
Males	19
Females	11
Prior chemotherapy	17
Performance status	
Median: 70	
Range: 40–100	
Tumor types	
Lung	11
Adenocarcinoma	6
Large cell carcinoma	3
Small cell carcinoma	1
Squamous cell carcinoma	1
Colorectal carcinoma	5
Breast carcinoma	3
Renal cell carcinoma	2
Malignant melanoma	1
Synovial sarcoma	1
Lymphocytic lymphoma	1
Leiomyosarcoma	1
Astrocytoma	1
Adenocarcinoma, endometrium	1
Nodular sclerosing Hodgkin's disease	1
Clear cell carcinoma, vaginal wall	1
Adenocarcinoma, unknown primary	1

Clinical Pharmacologic Studies

The results of these studies are summarized in figures 1 and 2. When given by rapid iv injection (fig 1), AZQ showed a rapid phase of drug distribution (α-half-life = 1.5 minutes) followed by a slower phase of drug elimination (β-half-life = 18 minutes). AZQ could not be detected in blood samples obtained at 24 hours after drug administration. The apparent volume of distribution for AZQ was calculated at 2.1 liters/kg. This large apparent volume of distribution is consistent with that usually associated with lipid-soluble drugs and implies wide distribution, extensive tissue uptake, or both. When AZQ was given by slow iv infusion over a period of 5 minutes (fig 2), the β-half-life was similar to the β-phase estimation for more rapid drug administration (fig 1). This indicates that the elimination phase is not dependent on the rate of drug administration. No metabolites were detected over the 60-minute period examined in this study. It is possible, however, that our method, designed to measure AZQ specifically, fails to detect metabolites that differ substantially from the parent drug with respect to solubility characteristics and chromatographic and spectroscopic properties (9). In one patient given a single iv dose of 15 mg/m², no AZQ was detected in the ventricular CSF obtained 60 minutes after injection of AZQ. Simultaneous serum samples revealed higher drug concentrations but α and β phases similar to those shown in figure 1.

TABLE 2.—Hematopoietic effects of AZQ in patients with little or extensive prior therapy

Dose level (mg/m^2/day × 5)	No. of courses	Median wbc count nadir, cells × $10^3/mm^3$ (range)	Median platelet count nadir, cells × $10^3/mm^3$ (range)
		Little prior therapy	
0.5	1	—	—
3.0	1	—	—
4.0	2	—	—
4.5	1	—	—
7.0	8	3.3* (0.9–8.5)	40* (16–240)
10.0	3	0.6 (0.5–0.7)	5 (2–16)
		Extensive prior therapy	
0.5	1	—	—
1.0	2	6.7 (4.9–8.5)	114 (58–171)
2.0	2	—	—
3.0	2	4.2 (3.5–5.0)	65 (18–112)
4.0	6	3.5 (2.2–3.7)	76 (26–330)
4.5	2	6.2 (3.6–8.6)	110 (109–112)
7.0	5	1.5† (0.9–2.0)	16† (2–68)
10.0	1	0.4	6

*Based on only 5 patients; 3 others had no changes.

†Based on only 4 patients; 1 other had no change.

DISCUSSION

AZQ was chosen for phase I trial because of its solubility characteristics and because of its activity in a number of murine tumor systems. In this phase I trial of AZQ, the toxicity (myelosuppression) was manageable and, in contrast to preclinical studies, no appreciable gastrointestinal toxicity was found. In addition, we found that the drug rapidly disappears from the serum in a biphasic manner, and in one patient the drug did not appear to penetrate the central nervous system. In view of the preclinical data and because other investigators have been able to detect AZQ in the CSF of nonhuman primates (6) and of patients (10,11) given the same or higher doses of this drug, tumors of the central nervous system should be included in phase II trial. Although we did not demonstrate the presence of AZQ in the single CSF specimen examined, that experience is insufficient, and additional studies of penetration of AZQ into CSF must be done. Since toxicity was restricted to myelosuppression, examination of AZQ in leukemia is warranted, and the limited activity seen in colon cancer in this phase I study suggests further trial in that tumor. On the basis of these toxicity data and the evidence of antitumor activity in one patient with colon cancer, we recommend that a phase II trial be initiated in solid tumors at an iv dose of 7.0 mg/m^2/day × 5 in patients with little prior chemotherapy and/or radiotherapy and at 4.0 mg/m^2/day × 5 in patients with extensive prior treatment. For patients with leukemia we recommend that further studies begin at an iv dose of 10 mg/m^2/day × 5.

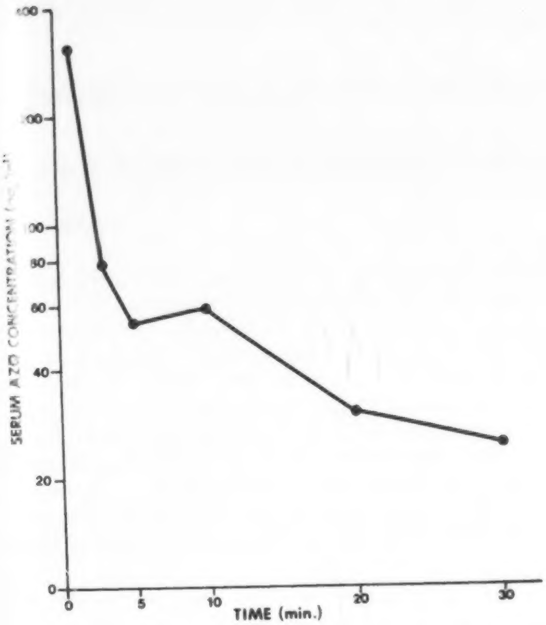

FIGURE 1.—Serum drug concentrations of a single patient given 7.0 mg/m² of AZQ by a rapid (0.5 min) iv injection. Data are presented as means of 3 determinations/time point.

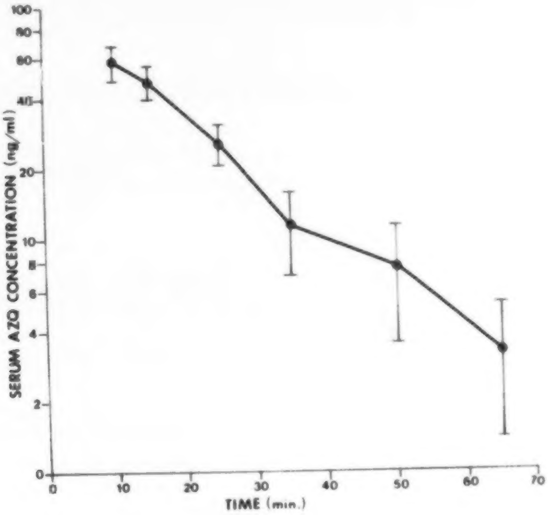

FIGURE 2.—Serum drug concentrations of 11 patients given 7.0 mg/m² of AZQ by a 5-min iv infusion. 9 patients received at least 2 courses of AZQ at this dose level. Data are presented as means ± SE of 11–20 determinations/time point.

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Clinical and Clinical Pharmacologic Studies of Mitoxantrone[1,2]

James A. Stewart,* John J. McCormack, and Irwin H. Krakoff[3,4]

SUMMARY

The drug mitoxantrone was given to 31 patients in a phase I clinical trial. The schedule of administration was once weekly for 3 weeks followed by 3 weeks of observation before further treatment. Dose range was from 1.5 to 6 mg/m². Leukopenia was the dose-limiting toxic effect and thrombocytopenia was mild. Partial alopecia occurred in four of 12 patients evaluable for hair loss. Pharmacokinetic studies employing a fluorometric assay based on DNA binding measured by displacement of ethidium bromide revealed a first-phase half-life of about 15 minutes, with a prolonged subsequent phase. A dose of 6 mg/m²/week × 3 was well-tolerated in patients not heavily pretreated with myelosuppressive therapy. Only minor clinical activity was seen with mitoxantrone.

[Cancer Treat Rep 66:1327–1331, 1982]

Mitoxantrone (DHAD) is a new antineoplastic drug developed in an attempt to improve on the clinical effectiveness of currently used anthracyclines (1). The irreversible myocardiopathy which can result from large cumulative doses of doxorubicin limits its use in patients with responsive tumors. In preclinical studies, DHAD has been shown to produce less cardiotoxicity but comparable antitumor effect (2).

Antineoplastic screening studies with DHAD showed activity in P388 and L1210 leukemia, B16 melanoma, and Colon 26 murine tumor systems. Studies in rats, beagle dogs, and cynomolgus monkeys determined that the major toxic effects of DHAD were diarrhea, emesis, and myelosuppression, all findings associated with many other clinically used anticancer agents. No evidence of cardiotoxicity was found clinically or on microscopic examination of myocardium in animals treated with DHAD (2).

MATERIALS AND METHODS

A phase I clinical study was undertaken to determine the toxicity of DHAD given as a single dose weekly for three doses. Pharmacologic studies were also done on some of the treated patients to determine the pharmacokinetics of DHAD and DHAD equivalents.

The patients treated in this study had histologically proven cancer that was not responsive to standard therapy or for which there was no conventional treatment with a reasonable expectation of benefit. Patients were treated in the ambulatory clinic at the Vermont Regional Cancer Center or in the wards of the Medical Center Hospital of Vermont. The histologic types of tumor are listed in table 1. Patient characteristics are shown in table 2.

Pretreatment evaluation included cbc with differential and platelet count, SMA-6 and -12 chemistry profiles, ECG, bone marrow aspirate and biopsy, urinalysis, and physical examination in all patients. Other procedures including plain X-rays necessary for clinical evaluation were done when indicated. Patients with a history of doxorubicin treatment or those who were considered at high risk

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[1] Received Aug 3, 1981; revised Dec 15, 1981; accepted Jan 5, 1982.

[2] Supported by contract N01-CM-97278 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Departments of Medicine and Pharmacology and the Vermont Regional Cancer Center, University of Vermont, Burlington.

[4] We thank Leslie Couser for her assistance with clinical data management.

**Reprint requests to:* James A. Stewart, MD, Vermont Regional Cancer Center, 1 S Prospect St, Burlington, VT 05401.

for development of congestive heart failure underwent radionuclide ventriculography to determine the status of myocardial performance. During evaluation each patient had cbc with differential and platelet count three times a week and urinalysis and chemistry profiles weekly.

Each patient was interviewed and examined prior to each dose of DHAD. Informed consent consistent with federal and University of Vermont guideline was obtained from each patient before entry in th study. No patients were treated with other chen otherapy within 30 days of beginning treatment wit DHAD.

DHAD was supplied by the Investigational Dru Branch of the National Cancer Institute (Bethesd MD) as a sterile solution at a concentration equi alent to 0.5 mg/ml of the free base. The drug wa diluted in 50 ml of 5% dextrose in water or norm saline and was injected through the side port of freely flowing iv line over 5–20 minutes.

The schedule of drug administration was a singl injection weekly for 3 weeks followed by 3 week of observation prior to the start of a new course One third of the toxic dose low in the beagle dog the most sensitive species, was chosen as the initial dose; the dose was 1.5 mg/m² for the first patients treated.

Dose escalations followed a modified Fibonacci scheme as outlined in table 3. At least three patients were treated at each dose level. Patients who demonstrated no prohibitive hematologic toxicity and no tumor progression during one course were retreated at the same or higher dose level. When a course of therapy consisted of less than three doses of DHAD a new course was not begun before 3 weeks following the last dose. Thirty-one patients were treated with 51 courses of DHAD, each course of treatment being one, two, or three doses of drug depending on toxicity.

TABLE 1.—Tumor types

Tumor	No. of patients
Non-small cell lung carcinoma	9
Breast carcinoma	7
Colon carcinoma	4
Carcinoma, unknown primary	3
Renal cell carcinoma	2
Ovarian carcinoma	1
Bladder carcinoma	1
Leiomyosarcoma	1
Rhabdomyosarcoma	1
Small cell lung carcinoma	1
Medullary thyroid carcinoma	1
	31

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Total	31
Male	14
Female	17
Median age in yrs (range)	
Male: 51.4 (29–67)	
Female: 59.4 (47–78)	
Prior treatment	
None	6
Chemotherapy	20
Doxorubicin to 400 mg/m²	7
Radiotherapy	19

Assay of DHAD and Equivalents

The fluorometric assay is carried out as follows: ethidium bromide (Sigma Chemical Co, St. Louis, MO; 50 µl of a 1×10^{-5} M solution in water) is added to pH 7.0 phosphate buffer (2.0 ml) and to this solution is added a sample of serum (usually, 100–200

TABLE 3.—Myelosuppression by DHAD

		Median wbc count (range)*		Median platelet count (range)*	
Dose (mg/m²)	No. of patients/ No. of courses	Before therapy	After therapy	Before therapy	After therapy
1.5 × 3	3/3	6.5	3.6 (2.7–5.6)	250	124 (117–162)
2.0 × 2	1/1	4.3	3.4	183	108
2.0 × 3	4/4	6.4	5.2 (3.3–11.9)	427	301 (53–595)
4.0 × 1	4/5	5.7	1.9 (1.6–2.8)	235	206 (60–224)
4.0 × 2	5/7	8.5	1.8 (1.0–2.6)	187	120 (70–195)
4.0 × 3	6/8	6.8	2.5 (1.6–5.3)	352	301 (99–455)
6.0 × 2	13/14	6.8	2.1 (1.2–6.4)	330	196 (47†–393)
6.0 × 3	7/11	10.2	3.8 (1.5–87.9)	311	200 (93–460)

*Cells × 10^3/mm³.

†Patients had thrombocytopenia prior to therapy.

μl); the resulting solution is used as a blank to zero the spectrophotofluorometer (Aminco-Bowman instrument; excitation, 476 nm; emission, 596 nm). DNA (Sigma, type I; 50 μl of a solution containing 2 mg of DNA in 50 ml of pH 7.0 phosphate buffer) is then added and the increment in fluorescence intensity at the indicated wavelength is measured. For serum samples containing DHAD, the expected increase in fluorescence on adding DNA is suppressed in a concentration-dependent manner. A standard curve was generated for each patient studied by adding increments of DHAD (5–50 μl of a 5-μg/ml stock solution in water) to a cuvette containing serum obtained prior to drug administration, buffer, ethidium bromide, and DNA and by recording the decrease in fluorescence that occurred with each increment of DHAD.

RESULTS

Toxicity

Myelosuppression was the major side effect noted, with leukopenia being dose-limiting. The median pretreatment platelet and wbc counts are shown in table 3. Most patients receiving a three-dose course of DHAD experienced a wbc count nadir at approximately Day 20 (median). For those patients treated with a course of 4 or 6 mg/m² × 3, the median granulocyte count nadir was 1.4 × 10^3 (range, 0.6–4.2). This nadir occurred at a median of 21 days (range, 15–26).

DHAD appears to be relatively platelet-sparing. In no patient was thrombocytopenia dose-limiting. The median nadir platelet count for each level and the number of doses was always > 50% of the median pretreatment level. In patients treated at the higher doses of DHAD, the degree of wbc suppression was dose-related.

Alopecia occurred in four of 12 patients who were evaluable for that effect. Nineteen patients were not evaluable because of prior chemotherapy or radiotherapy to the scalp which caused alopecia. Of the four patients who had hair loss during DHAD therapy, none had total alopecia. One patient who had no hair loss during DHAD treatment (4.0 mg/m² × 3 and 6.0 mg/m² × 2) did experience partial alopecia with subsequent doxorubicin administration at 60 mg × 1. Another patient who developed alopecia during a course of cyclophosphamide, cisplatin, and doxorubicin noted regrowth of hair during DHAD therapy (6 mg/m² × 2).

Nausea was very mild or absent in all patients. No patient had reproducible vomiting that could be related to DHAD administration. One patient did experience a brief episode of vomiting 20 hours after her second dose of 4.0 mg/m². Five patients had mild nausea for about 24 hours after DHAD injection. Two patients complained of feeling cold and tired for 24–48 hours after each dose of DHAD.

At the doses given, no patient had blue discoloration of the sclerae or skin. Urine voided soon after drug administration was frequently noted to have a green color.

No patient had obvious cardiotoxicity secondary to DHAD. The largest cumulative doses in this study were 45 and 36 mg/m². One patient who had received 470 mg/m² of doxorubicin developed biventricular cardiac failure approximately 2 years after cessation of doxorubicin therapy. The signs and symptoms of congestive heart failure were well-controlled with digoxin and diuretics prior to therapy with DHAD. Treatment with DHAD to a total of 45 mg/m² was not associated with clinical worsening of his heart failure.

Response

Two patients exhibited brief clinical responses to DHAD. One woman with metastatic breast cancer previously treated with multiple courses of cytotoxic and hormonal therapy had an objective decrease in the size of numerous small skin metastases. For individually measured lesions the area of tumor reduction was < 50% and within 7 weeks she had bone disease manifested by pain and radiographic changes.

Another patient with metastatic breast cancer had a marked decrease in pain after 2 days of increased pain coincident with DHAD therapy. Her disease was metastatic to the bones and this symptomatic response was identical to that seen with her first treatment with cyclophosphamide, methotrexate, and 5-FU which led to a 1-year partial remission. Despite the initial symptomatic improvement with DHAD, however, she had definite disease progression within 6 weeks and later failed to respond to doxorubicin.

No patients with lung cancer, including seven who had received no prior chemotherapy, showed a response to DHAD.

Pharmacologic Studies

At the time these studies with DHAD were initiated there were no published methods available for analytic studies of this drug in biologic samples. The development and application of high-performance liquid chromatography (HPLC) procedures for DHAD analysis were unsatisfactory and a different approach to analysis was therefore sought. The approach is based on the ability of DHAD to form intercalative complexes with DNA and involves measurement of the decrease in fluorescence of DNA-ethidium bromide complexes that occurs when DHAD displaces ethidium bromide from the complex. As shown in table 4, when samples of human serum containing DHAD are analyzed by this method a concentration-dependent suppression of fluorescence is observed. The method is capable of detecting the presence of DHAD in serum (and urine), even at the lowest drug dose studied (1.5 mg/m²). Most of these studies of DHAD levels in serum were carried out on samples obtained when the drug was administered at 6 mg/m². Table 5 summarizes these studies; DHAD levels decrease rapidly (estimated half-life = 15 minutes) during the first 30 minutes after completion of drug infusion with a subsequent much slower phase. There is a consistent increase in DHAD (and/or equivalents) when serum levels are analyzed 24 hours after drug administration. This observation may reflect redistribution of DHAD from tissue "stores" or the formation of a DHAD metabolite that is more potent than the parent compound as an intercalator and therefore is detected by this assay as more than one DHAD equivalent. Further studies of the later stage(s) of DHAD elimination from serum clearly are indicated and we plan to carry out such studies, both in experimental animals and in patients participating in phase II studies with DHAD. It is important to note that the "peak" drug levels observed in this study were comparable, taking into account different techniques of administration (ie, infusion versus bolus injection) to those reported recently for a somewhat higher dose of DHAD in man, using ^{14}C-labeled drug and HPLC analysis (3). In addition, studies with the labeled drug also revealed the presence of significant levels of DHAD in the blood at 24 and 72 hours after iv administration of this drug. The estimated initial half-life (rapid phase) for DHAD as well as the suggestion of a prolonged elimination phase that follows the rapid elimination phase are in agreement with observations on the clinical pharmacology of this drug reported by Savaraj et al (4); similar elimination profiles are observed with doxorubicin in patients and in experimental animals (5,6).

TABLE 4.—Inhibition of fluorescence by DHAD in human serum samples*

Concentration of DHAD (μM)	% inhibition of fluorescence
0.15	86.3 ± 6.2
0.10	71.1 ± 4.0
0.075	54.6 ± 3.6
0.050	43.3 ± 3.6
0.025	22.8 ± 2.8

*N = 8. Serum samples were obtained from patients before administration of DHAD. The indicated amounts of DHAD were added to assay solutions containing 100–200 μl of serum.

DISCUSSION

This trial demonstrates that DHAD can be given to patients who have not been heavily pretreated with other chemotherapy at a dose of 6 mg/m² weekl for three doses. Reversible myelosuppression is th major toxic effect and there appears to be a relative platelet-sparing effect. Nonmyelosuppressive toxicity was mild at this dose; however, partial alopecia did occur in some patients.

There is laboratory evidence that the mechanism of the antineoplastic action of DHAD is at least in part similar to that of doxorubicin (7). Both drugs bind to DNA and inhibit nucleic acid synthesis. DHAD, however, may have antineoplastic activity on the basis of effects other than DNA intercalation.

If the lack of the amino sugar moiety eliminates or lessens cardiotoxicity for DHAD compared to doxorubicin, then clinical utility will be greatly enhanced. We are unable to comment on the presence or lack of DHAD-induced heart damage but it must be noted that the highest cumulative doses attained in this study do not approach those which would be expected to cause cardiac toxicity. Long-term use will approach such levels and continued evaluation will be necessary.

Patients with a history of prior doxorubicin therapy may develop cardiotoxicity during subsequent DHAD therapy. Several cases of suspected DHAD-induced heart disease have occurred in ongoing phase II trials. Because of the difficulty in determining the etiology of cardiac toxicity in patients who have received both doxorubicin and DHAD, investigators should follow the recommendations of the Investigational Drug Branch of the National Cancer

TABLE 5.—Serum concentration of DHAD (and/or equivalents)

Time*	Concentration (μM)†
0	0
5 mins	0.47 ± 0.06 (0.75)
15 mins	0.31 ± 0.03 (0.50)
30 mins	0.19 ± 0.02 (0.21)
60 mins	0.16 ± 0.03 (0.12)
120 mins	0.15 ± 0.03
24 hrs	0.22 ± 0.04 (0.21)

*"0" is immediately before infusion began; other times are following completion of infusion.

†Includes DHAD and any metabolites that are capable of intercalating into DNA; mean ± SEM. Nos. in parentheses are for a patient given DHAD at 12 mg/m² for pharmacologic study; all other values are for 11 patients given 6 mg/m².

I stitute and restrict eligibility for DHAD therapy t those patients without a history of heart disease a d who have not received doxorubicin at a cumul ve dose > 400 mg/m² for adults and 450 mg/m² f children.

n a phase I study in which DHAD was given as a ngle dose every 3 weeks, Von Hoff et al (8) found 1 mg/m² to be the maximum tolerated dose. They, t , failed to see cardiac toxicity, but in their study a o, cumulative doses did not reach levels which w l be seen with prolonged therapy and which might b expected to cause myocardiopathy. Other phase I rials of DHAD using schedules of weekly (9), daily × 3 every 3 weeks (10), and daily × 5 every 3 weeks (11) drug administration have been reported. In these trials myelosuppression was also the dose-limiting toxic effect.

On the basis of promising preclinical data and good patient tolerance of the drug we would recommend further studies of DHAD, particularly in patients who have not been treated with doxorubicin, to determine cardiotoxicity and antitumor effectiveness. A weekly dose of 6 mg/m² for 3 weeks is a suitable schedule for patients without extensive prior chemotherapy or radiotherapy.

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Plasma and Cerebrospinal Fluid Pharmacokinetics of Acivicin in Ommaya Reservoir-Bearing Rhesus Monkeys[1]

J. Patrick McGovren,[2,*] Julianna C. Stewart,[2] Gary L. Elfring,[2] Randall B. Smith,[2] Nirmolini Soares,[3] James H. Wood,[4] David G. Poplack,[3] and Daniel D. Von Hoff[3,5,6]

SUMMARY

Acivicin was administered iv to rhesus monkeys bearing Ommaya reservoirs, and serial blood and cerebrospinal fluid (CSF) samples were collected and analyzed to determine the time course of drug concentrations in these body fluids. After iv doses of 4 or 20 mg/kg (50 or 250 mg/m^2), acivicin plasma concentrations demonstrated a rapid initial decline (distribution phase), and then declined exponentially with a terminal (elimination phase) half-life of 3–4 hrs. CSF concentrations increased over a period of 2–2.5 hrs, reaching peak values of 2.0–2.7 μg/ml at 20 mg/kg and 0.3–0.5 μg/ml at 4 mg/kg; thereafter, CSF levels declined in parallel with plasma, with a CSF/plasma concentration ratio of 0.10–0.17. A three-compartment pharmacokinetic model gave a close fit of predicted and observed plasma and CSF concentration data. Significant and predictable CSF penetration by iv administered acivicin in monkeys is consistent with observation of CNS side effects in patients in the phase I clinical trial and suggests that acivicin should be evaluated in the treatment of CNS malignancies and metastases.

[Cancer Treat Rep 66:1333–1341, 1982]

Acivicin is an amino acid antitumor agent that is currently in phase I clinical trials (1–3). Recently, a report appeared describing CNS toxicity (dysphoria, lethargy, somnolence, ataxia, and hallucinations) as being dose-limiting in patients receiving a single dose every 3 weeks (4). CNS toxicity that was not dose-limiting was also observed in a daily dosing schedule (5). The only CNS effects observed in the dog and monkey toxicity testing done prior to the clinical trial were sedation and disorientation occurring at lethal and superlethal doses that caused death from gastrointestinal toxicity (6).

Acivicin was previously shown to cause significant prolongation of life in mice bearing intracranially inoculated L1210 leukemia (7) and ependymoblastoma (8). During extensive investigations of acivicin disposition and pharmacokinetics, the agent was measured in significant quantities in brain tissue of mice and dogs after parenteral administration (9; J. P. McGovren, unpublished data). Significant distribution of acivicin into brain tissue was unexpected because of the water-soluble, zwitterionic nature of the molecule at physiologic pH.

These findings prompted a kinetic study of the penetration of acivicin into the cerebrospinal fluid (CSF) in Ommaya reservoir-bearing rhesus monkeys. The Ommaya reservoir is a subcutaneously implanted device allowing direct access to the fourth ventricle of the brain for serial CSF sampling or drug administration in unanesthetized animals without disruption of the blood-brain barrier (10–12). The goal of the study was to quantitate the

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[1] Received Aug 19, 1980; revised Dec 17, 1981; accepted Jan 1982.

[2] Cancer Research, The Upjohn Co, Kalamazoo, MI.

[3] Pediatric Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4] Division of Neurosurgery, School of Medicine, University of Pennsylvania, Philadelphia.

[5] *Present address:* Division of Oncology, Department of Medicine, University of Texas Health Sciences Center, San Antonio.

[6] We gratefully acknowledge the valuable discussions on pharmacokinetic modeling with Carl M. Metzler.

*Reprint requests to: J. Patrick McGovren, PhD, Cancer Research, The Upjohn Co, Kalamazoo, MI 49001.

time course of acivicin concentrations in the plasma and CSF of an animal model thought to be predictive for the pharmacokinetic behavior of chemotherapeutic drugs in man (12). The findings should be helpful in understanding the CNS effects observed in humans receiving acivicin and in the clinical evaluation of acivicin activity against CNS malignancies and metastases. Such tumors are notably refractory to chemotherapeutic attack due (at least in part) to restricted passage of drugs across the blood-brain barrier (13).

MATERIALS AND METHODS

Acivicin was produced by microbial fermentation and purified at The Upjohn Company (14–16).

Adult male rhesus monkeys (from the NIH Primate Center) weighing 5.4–8.6 kg and bearing previously implanted Ommaya reservoirs were used in the studies. Details of animal preparation and care were as previously described (10–12). Integrity of the reservoir system was monitored by biweekly cell counts and cultures on 3-ml CSF samples.

Pharmacokinetic Studies

Monkeys received doses of 20 or 4 mg/kg (250 or 50 mg/m^2). Two monkeys were studied at each dose. Acivicin was administered as a bolus into the saphenous vein as the appropriate volume of 10 mg/ml of drug in 0.9% NaCl solution. Blood and CSF samples were drawn before treatment and at 15, 30, and 45 mins and 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 9, 12, 24, 28, and 32 hrs after dosing. CSF was withdrawn from the reservoir as previously described (11). Blood samples were taken from the cephalic vein and centrifuged; plasma was then harvested and frozen until assay. To monitor possible drug-related toxicity, blood samples were also taken before treatment and at weekly intervals for the following determinations: cbc including platelet count, prothrombin time, partial thromboplastin time, serum glutamic-pyruvic transaminase, sodium, potassium, chloride, and SMA/12 serum chemistry profile.

Analytic Methods

Acivicin concentrations in monkey plasma and CSF were assayed by a microbiologic method using *Bacillus subtilis* UC-902 cultivated in a completely synthetic medium (16). Samples were assayed full strength or diluted in 0.1 M Na_2HPO_4/NaH_2PO_4 buffer. Standards were prepared by the addition of known quantities of acivicin to drug-free plasma or CSF at the same dilution as the samples. Aliquots of samples and standards were applied to paper discs (Schleicher and Schuell Co, Keene, NH) on agar trays seeded with the microorganism. A complete set of six standards (ranging from 0.059 to 0.44 µg/ml) was applied to each tray; each sample and standard was applied in triplicate to each tray. Trays were incubated at 37°C for 15–18 hrs, and the zones of inhibition were measured to the nearest millimeter. Standard curves were constructed by plotting the log concentration of the standards versus zone size. Standard curves in plasma diluted 1:200 to 1:30 and in CSF at all dilutions were straight lines; a statistical least-squares linear regression program for a programmable calculator was used to fit equations to the lines and interpolate the sample values. Standard curves in plasma at lower dilutions were curvilinear and sample values were visually interpolated. Most samples were assayed at two dilutions and the results were averaged. Samples with concentrations falling outside of the standard curve range were reassayed at different dilutions.

Bioautographic Studies

Bioautographic studies were performed to determine the specificity of the microbiologic assay. Aliquots of selected plasma and CSF specimens containing previously assayed amounts of acivicin bioequivalents were applied to plastic-backed thin-layer chromatography sheets (Baker-flex IB-F flexible sheets, J. T. Baker Chemical Co, Phillipsburg, NJ) along with spiked samples containing known amounts of authentic acivicin. The volume applied was < 10 µl. After sample application, the plates were dried in a stream of warm air and developed in tetrahydrofuran:acetone:water (5:3:2), methylethyl ketone:acetone:water (2:1:1), acetone:water (8:2), or isopropanol:28.5% ammonium hydroxide:water (7:1:2). Following development, the sheets were dried in a stream of warm air for 3 hrs. The silica gel surface of the sheets was then carefully placed in contact with agar trays seeded with *B. subtilis* (synthetic medium) for 30 mins. The sheets were peeled off and discarded, and the trays were incubated. The resulting zones of growth inhibition for samples were then used to calculate R_f values, which were compared to those of acivicin standards. Only one zone of inhibition per sample was observed in all bioautographic studies, and in each case the R_f of the material corresponded to that of intact acivicin. Therefore, all pharmacokinetic calculations were done with the assumption that only intact acivicin was measured by the assay.

Pharmacokinetic Modeling

To define the dynamic relationship between the CSF and plasma concentrations, compartmental pharmacokinetic modeling was carried out on the data. The triexponential equations for the time course of drug concentrations in the three-compartment open model for drug disposition (17) were curve-fitted simultaneously to the plasma and CSF concentrations of each monkey (fig 1).

In this model, the central compartment consists of plasma and rapidly perfused tissues and the peripheral compartment consists of poorly perfused tissues. The "brain" compartment is defined as containing CSF and brain extracellular and intracellular spaces, all of which are assumed to be in rapid equilibrium. Elimination by metabolism and excretion is assumed to occur only from the central compartment. Metabolism of acivicin within the brain is certainly possible, but the data are not sufficient to allow incorporation of this aspect into the pharmacokinetic model.

The equations describing the three-compartment model and additional calculated parameters are given in the Appendix. The coefficients, exponents, and compartmental volumes in the integrated equations were estimated by simultaneous iterative curve fitting of the plasma and CSF concentrations using the nonlinear least-squares parameter estimation program, NONLIN, on an IBM 370/158 digital computer (18). Initial estimates of fitted parameters were obtained by manual feathering techniques (17). To minimize the difference in magnitude between the plasma and CSF concentrations for computer fitting, the data were weighted using the factor, $1/yi^2$. That is, the following function was minimized:

$$\sum_{i=1}^{N} 1/y_i^2 \, (yi_{obs}-yi_{calc})^2,$$

where yi_{obs} is the observed value of the *i*th measurement, yi_{calc} is the calculated or fitted value of the *i*th measurement, and N is the number of data points. This weighting approach is statistically valid because all plasma and CSF samples were diluted to the same narrow concentration range prior to assay (19,20).

Attempts were made to fit the three-compartment model, with all rate constants and volumes (including V_3) allowed to vary and with V_3 fixed at the approximate anatomic volume of the brain in a monkey, 0.03 liter/kg (21). Goodness of fit was determined from correlation coefficients (r^2), residual sums of squares, standard deviations of estimated parameters, and visual inspection of plots of the predicted and observed concentrations versus time.

RESULTS AND DISCUSSION

No adverse effects of acivicin administration at either dose were observed upon daily physical examination or in the hematology and blood chemistry studies. Although no previous single-dose toxicity studies had been conducted in monkeys, 2.6 mg/kg/day on the daily × 5 schedule was determined to be a lethal dose (6).

Figure 2 shows plasma and CSF concentrations of acivicin in monkeys receiving single 4- or 20-mg/kg doses iv. Plasma concentrations declined in a multiexponential fashion, with a terminal (elimination phase) half-life of 3–4 hrs. The initial (distribution) phase in plasma appeared to be complete by approximately 1 hr after dosing. As indicated by the ratio of the areas under the plasma level curves (AUC_1) at the two doses (table 1), plasma concentrations were proportional to the doses administered. CSF concentrations also appeared to be proportional to the dose. CSF concentrations increased over a period of 2–2.5 hrs and then declined in parallel to plasma levels with CSF/plasma concentra-

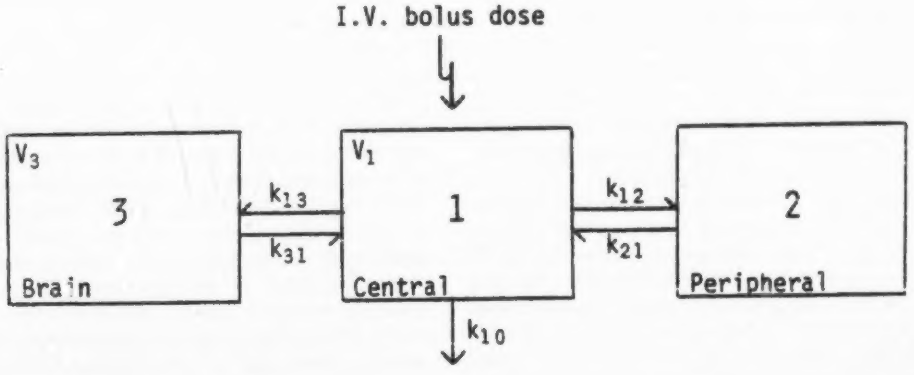

FIGURE 1.—The 3-compartment open model for acivicin disposition.

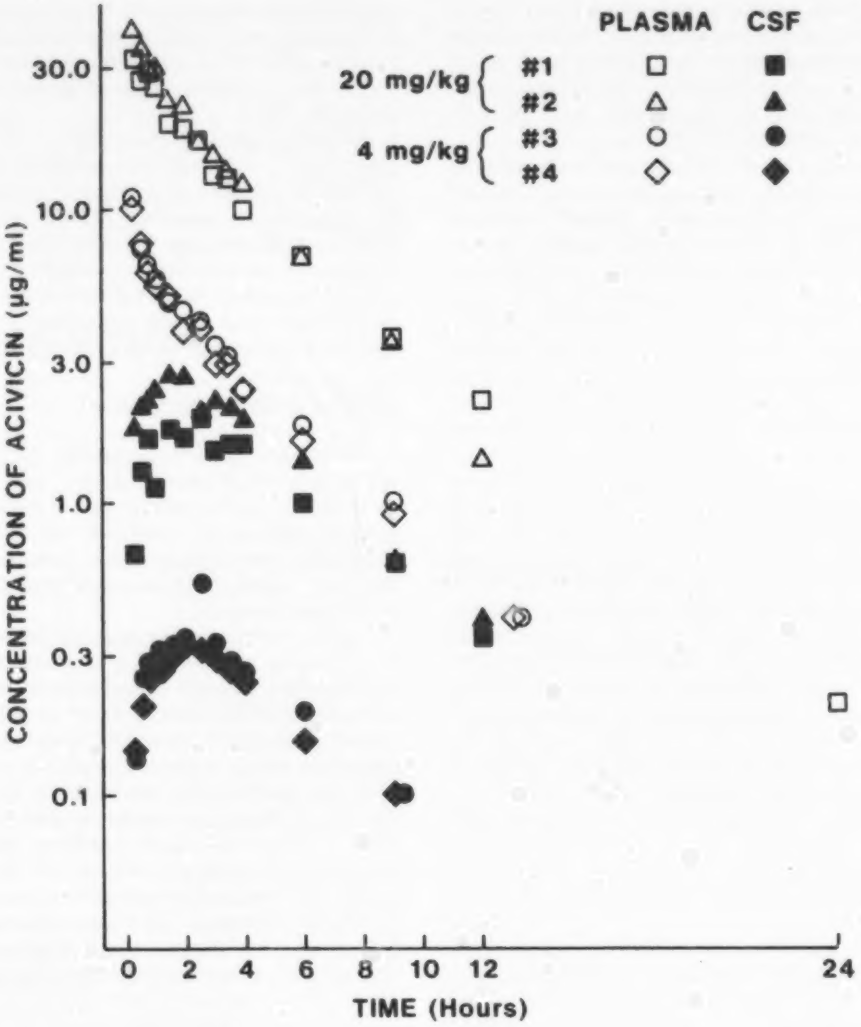

FIGURE 2.—Time course of plasma and CSF concentrations of acivicin following 20- and 4-mg/kg iv doses in 4 rhesus monkeys.

tion ratios of 0.10–0.17 in the postdistributive phase. CSF concentrations > 1.0 µg/ml were maintained for almost 6 hrs at the 20-mg/kg dose.

Free diffusion of acivicin, a water-soluble amino acid in the zwitterionic form at physiologic pH, across the blood-brain barrier was not expected to occur. The observed degree of uptake into CSF from blood can, therefore, be attributed to some type of transport process, as has been postulated for other neutral amino acids (22).

On initial examination, the data appeared to be suitable for description by a two-compartment open model for drug disposition. However, the initial rapi decline in plasma concentrations (distribution phase was complete by approximately 1 hr after dosing whereas CSF concentrations did not peak and begi to decrease in parallel with the plasma levels unti 2–3 hrs. This feature of the data is inconsistent wit the two-compartment model and implies that in addition to the partitioning into CSF, drug is distributing from plasma into a compartment kinetically distinct from that containing the CSF. Therefore, a three-compartment model is necessary to describe the data. Since CSF and brain are separated from

TABLE 1.—Fitted and derived pharmacokinetic parameters of 3-compartment model for iv administered acivicin in plasma and CSF of rhesus monkeys*

Parameters	Monkey No.				Mean	SD
	1	2	3	4		
Dose (mg/kg)	20	20	4	4		
Weight (kg)	5.4	6.1	7.6	8.6	6.9	1.4
P	0.43	0.35	0.65	0.58	0.50	0.14
A	0.0023	0.0052	0.00084	0.0023	0.0027	0.0018
B	0.57	0.65	0.35	0.41	0.50	0.14
P'	–0.0016	–0.0020	–0.0012	–0.0013	–0.0015	–0.00036
A'	–0.0039	–0.0069	–0.0051	–0.0039	–0.0050	–0.0014
B'	0.0055	0.0090	0.0063	0.0052	0.0065	0.0017
π (hr^{-1})	1.91	2.36	4.38	3.06	2.93	1.08
α (hr^{-1})	0.67	0.89	0.61	0.65	0.71	0.13
β (hr^{-1})	0.20	0.26	0.23	0.23	0.23	0.02
$t_{1/2_\pi}$ (hr)	0.36	0.29	0.16	0.23	0.26	0.09
$t_{1/2_\alpha}$ (hr)	1.03	0.78	1.14	1.07	1.01	0.16
$t_{1/2_\beta}$ (hr)	3.47	2.67	3.01	3.01	3.04	0.33
V_1 (liters/kg)	0.47	0.39	0.20	0.27	0.33	0.12
V_B (liters/kg)	0.78	0.57	0.52	0.57	0.61	0.12
k_{10} (hr^{-1})	0.33	0.38	0.60	0.49	0.45	0.12
k_{12} (hr^{-1})	0.61	0.60	2.32	1.38	1.23	0.81
k_{21} (hr^{-1})	1.17	1.63	1.69	1.40	1.47	0.24
k_{13} (hr^{-1})	0.0046	0.0088	0.0024	0.0066	0.0056	0.0027
k_{31} (hr^{-1})	0.67	0.88	0.61	0.65	0.70	0.12
AUC_1 (mg-hr/liter)	129.4	136.5	32.6	30.5		
AUC_3 (mg-hr/liter)	13.8	17.1	2.5	2.2		
Cl_B (ml/hr)	837	904	912	1138	948	131
Cl_{31} (ml/hr)	109	161	139	168	144	27

*Volume of distribution of brain compartment, V_3, was fixed at the estimated anatomic volume of CSF in a monkey, 0.03 liter/kg, for computer fitting of data from all animals. All other constants and volumes were allowed to vary or "float." For definition of parameters, *see* the Appendix.

the general circulation and other "peripheral" tissues by the blood-brain barrier, the three-compartment model is also appropriate from an anatomic standpoint. Although the fitting program converged to a solution, large standard deviations estimated parameters resulted when all rate con- ants and volumes (including that of the brain com- rtment) were allowed to vary or "float" in the ting program. In subsequent fitting, V_3 was fixed the approximate anatomic brain volume of a mon- y, 0.03 liter/kg (21). This assumption was sup- rted by the computer estimates of V_3 obtained r the four monkeys when all constants and vol- nes were varied: 0.030, 0.031, 0.011, and 0.037 er/kg.

The plasma concentration-time course data do not ave the triphasic appearance typical of data that re appropriately described by a three-compartment model, apparently because the volume of the compartment containing CSF was quite low compared to that of the central compartment; ie, the actual amount of drug in the "brain" compartment at any time was very small relative to that in the rest of the system, and there was no detectable effect of that particular distribution process on the kinetics in plasma. Fitting the plasma and CSF data simultaneously to a three-compartment model allowed reasonable estimates of the central-CSF distribution constant, π, to be made from the CSF data (which contained insufficient information on the central-peripheral distribution) and of the central-peripheral distribution constant, α, to be made from the plasma data (which contained insufficient information on the plasma-CSF distribution).

Table 1 summarizes the fitted and derived parameters of the three-compartment model describing acivicin kinetics in plasma and CSF of monkeys. Figure 3 shows plots of the predicted and observed plasma and CSF concentrations versus time for the two monkeys having the best and worst fits of the four sets of data analyzed. There was excellent agreement between the predicted and observed curves as demonstrated by the visual fit, the r^2 values, and the standard deviations of estimated pa-

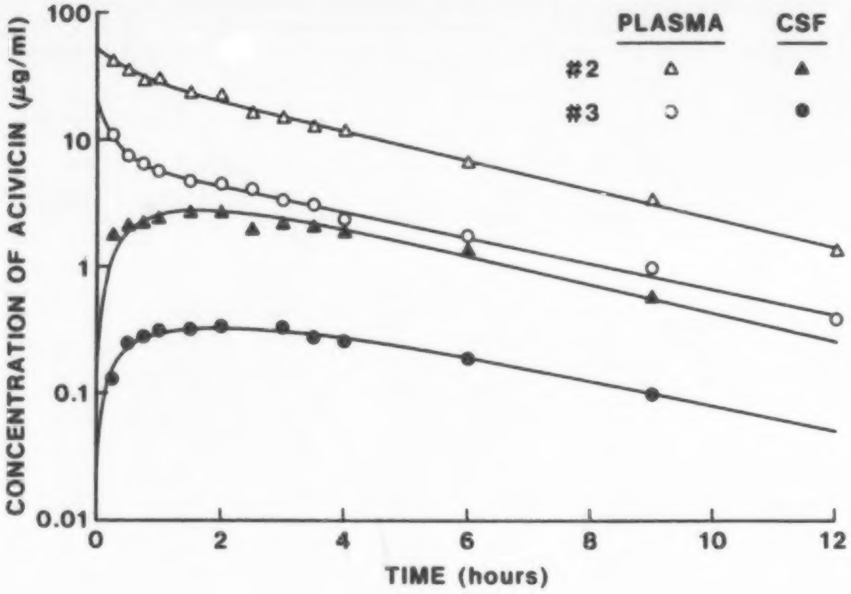

FIGURE 3.—Predicted and observed plasma and CSF concentrations of acivicin in 2 monkeys having the best (monkey No. 3) and worst (monkey No. 2) fits of the 3-compartment pharmacokinetic model to the 4 sets of data in fig 1. Lines were generated from equations describing the 3-compartment model using computer-estimated "best-fit" parameters for each animal (table 1).

rameters. The magnitude of k_{13} relative to the other transfer constants reflects the small mass of material entering the CSF compartment at any time. There appeared to be some dose dependency of the parameters V_1 and k_{12}.

The calculated value of the CSF-to-plasma intercompartmental clearance, $Cl_{31} = k_{31} \cdot V_3$, was dependent on the assumption that acivicin was distributed throughout the total brain volume and that all parts of the brain were in rapid equilibrium. As such, the absolute magnitude of Cl_{31}, 144 ml/hr, may not be directly comparable to measured CSF clearances of several radiolabeled endogenous amino acids determined by ventriculocisternal perfusions in cats (23,24), which were 2–3 ml/hr, since distribution of the amino acids beyond the CSF into brain tissue was low. However, the magnitude of Cl_{31} relative to the estimated CSF flow rate of 4–6 ml/hr in the rhesus monkey (25) indicates that acivicin was cleared from CSF at a much faster rate than would be expected from bulk flow alone. Therefore, acivicin was apparently rapidly transported out of the CSF. Metabolism of acivicin within the brain could also contribute to its rapid clearance, but there were not sufficient data to allow estimation of a brain metabolism constant in the pharmacokinetic model.

Most substances in CSF are freely available to brain parenchyma (13). Acivicin concentrations in whole-brain homogenate were previously measured as a function of time in mice and dogs and found to be from 20% to 100% of simultaneous plasma concentrations (9; J. P. McGovren, unpublished data). It is not known at this time whether the CNS effects observed in humans receiving iv administered acivicin are due to the intact molecule or to a metabolite, but the data suggest that acivicin itself could be available to elicit such effects. Whether these concentrations of acivicin in human CSF would result in brain levels that would be cytotoxic to a malignant growth is also not known at this time, but clinical evaluation of the agent against CNS malignancies and metastases should be considered.

APPENDIX

Gibaldi and Perrier (17) give the integrated equation describing the drug concentration in the three-compartment open pharmacokinetic model (C_1) as:

$$C_1 = \frac{X_0}{V_1}\left[Pe^{-\pi t}+Ae^{-\alpha t}+Be^{-\beta t}\right]$$

where

$$P = \frac{(k_{21}-\pi)(k_{31}-\pi)}{(\pi-\alpha)(\pi-\beta)}$$

$$A = \frac{(k_{21}-\alpha)(\alpha-k_{31})}{(\pi-\alpha)(\pi-\beta)}$$

$$B = \frac{(k_{21}-\beta)(k_{31}-\beta)}{(\alpha-\beta)(\pi-\beta)}$$

The equation for the drug concentration in the CSF compartment (C_3) was derived using the Laplace transform method described by Gibaldi and Perrier (17):

$$C_3 = \frac{X_0}{V_3}\left[P'e^{-\pi t}+A'e^{-\alpha t}+B'e^{-\beta t}\right]$$

where

$$P' = \frac{k_{13}(k_{21}-\pi)}{(\pi-\alpha)(\pi-\beta)}$$

$$A' = \frac{k_{13}(k_{21}-\alpha)}{(\pi-\alpha)(\beta-\alpha)}$$

$$B' = \frac{k_{13}(k_{21}-\beta)}{(\alpha-\beta)(\pi-\beta)}$$

and

$$k_{31} = 1/2\left[-b-\sqrt{b^2-4ac}\right]$$

$$k_{13} = 1/2\left[-b+\sqrt{b^2-4ac}\right]$$

where

$$a = 1$$

$$b = -\frac{\pi B+\pi A+\beta P+\beta A+\alpha P+\alpha B}{P+A+B}$$

$$c = \frac{\alpha\pi B+\pi\beta A+\alpha\beta P}{P+A+B}$$

$$k_{10} = \frac{\alpha\beta\pi}{k_{21}k_{31}}$$

$$k_{12} = \frac{(\alpha\beta+\alpha\pi+\beta\pi)-k_{21}(\alpha+\beta+\pi)-k_{10}k_{31}+k_{21}^{2}}{k_{31}-k_{21}}$$

$$k_{21} = \alpha+\beta+\pi(k_{10}+k_{12}+k_{21}+k_{31}).$$

C_n is the concentration of acivicin in compartment n; t is time; P, A, B, P′, A′, and B′ are coefficients; and α, β, and π are hybrid rate constants describing the concentration-time curves; X_0 is the dose; k_{10} is the elimination rate constant (sum of individual rate constants for all routes of elimination); k_{12}, k_{21}, k_{13}, and k_{31} are intercompartmental transfer rate constants; and V_1 and V_3 are the apparent distribution volumes of the central and CSF compartments.

Additional quantities calculated from the fitted parameters were:

$$V_B = \frac{X_0}{\beta\int_0^\infty C_1 dt} = \frac{V_1 k_{10}}{\beta}$$

$$AUC_1 = \int_0^\infty C_1 dt = \frac{X_0}{V_1}\quad \frac{P}{\pi} + \frac{A}{\alpha} + \frac{B}{\beta}$$

$$AUC_3 = \int_0^\infty C_3 dt = \frac{X_0}{V_3}\quad \frac{P'}{\pi} + \frac{A'}{\alpha} + \frac{B'}{\beta}$$

$$Cl_B = V_B \cdot \beta = V_1 \cdot K_{10}$$

$$Cl_{31} = V_3 \cdot k_{31},$$

where V_B is the apparent volume of distribution relating the plasma concentration to the total amount of drug in the body during the postdistribution phase; AUC_1 and AUC_3 are the total areas under the plasma and CSF concentration-time curves; Cl_B is the total body clearance of acivicin by metabolism and excretion; and Cl_{31} is the drug clearance from the CSF compartment.

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Studies on the *O*-Demethylation of Misonidazole by Rat Liver Microsomes[1]

D. D. Shoemaker,* M. E. McManus, R. Hoerauf, and J. M. Strong[2]

SUMMARY

The role of rat liver microsomes in the *O*-demethylation of misonidazole to desmethylmisonidazole was studied. The rate of the microsomal-dependent formation of desmethylmisonidazole was linear up to a protein concentration of 2 mg/ml and over a 10-minute interval. The metabolism was optimal in a system comprised of microsomes, O_2, and NADPH. Metabolism in incubation mixtures continuously flushed with N_2 was inhibited by 78%. The *O*-demethylase activity was competitively inhibited by the addition of SKF 525-A, with a Ki of approximately 1×10^{-5} M. The Km and Vmax values of normal microsomes were 1.87 ± 0.30 mM and 413 ± 14 pmols/minute/mg of microsomal protein, respectively. Pretreatment of rats with phenobarbital for 7 days prior to preparation of the microsomes resulted in no significant change in the Km, but the Vmax was considerably increased to 1033 ± 203 pmols/minute/mg of microsomal protein. The results demonstrate that the *O*-demethylation of misonidazole is mediated by cytochrome P-450.

[Cancer Treat Rep 66:1343–1347, 1982]

Misonidazole (fig 1), a hypoxic cell radiosensitizer presently undergoing clinical trials, is known to be extensively metabolized in rodents and man (1). The primary metabolite identified to date is desmethylmisonidazole and is formed by *O*-demethylation (1). Desmethylmisonidazole has been shown to be effective as a radiation sensitizer in vitro (2) and has been entered into phase I clinical trials (3). During clinical trials, misonidazole was observed to be associated with neurotoxicity (4), the incidence of which was lessened by concomitant therapy with inducers of the hepatic drug-metabolizing enzymes (5,6). In vivo studies in the mouse, the dog, and man demonstrated that pretreatment with known microsomal enzyme inducers increased both the plasma clearance of misonidazole and the percent of the dose excreted as the demethylated metabolite (7–9). No previous attempt has been made to study the conversion of misonidazole to the demethylated metabolite in the in vitro system. Therefore, the present studies of the *O*-demethylation of misonidazole to desmethylmisonidazole by liver microsomes prepared from normal and phenobarbital (PB)-pretreated rats were undertaken to characterize this metabolic reaction.

MATERIALS AND METHODS

Misonidazole was obtained from the National Cancer Institute (Bethesda, MD). Materials used in this study were: glucose-6-phosphate, glucose-6-phosphate dehydrogenase, NADH, and NADPH (Boehringer Mannheim Biochemicals, Indianapolis, IN); and phenobarbital sodium (Fisher Scientific Co, Fair Lawn, NJ). All other chemicals were of analytic reagent grade.

Male Sprague-Dawley rats (175–225 g, Taconic Farms, Germantown, NY) maintained on a diet of water and Purina Rat Chow (Ralston Purina Co,

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[1] Received July 6, 1981; revised Nov 17, 1981; accepted Dec 2, 1981.

[2] Laboratory of Chemical Pharmacology, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD.

**Reprint requests to:* Dr. D. D. Shoemaker, Investigational Drug Branch, National Cancer Institute, National Institutes of Health, Landow Bldg, Rm 4C19, 7910 Woodmont Ave, Bethesda, MD 20814.

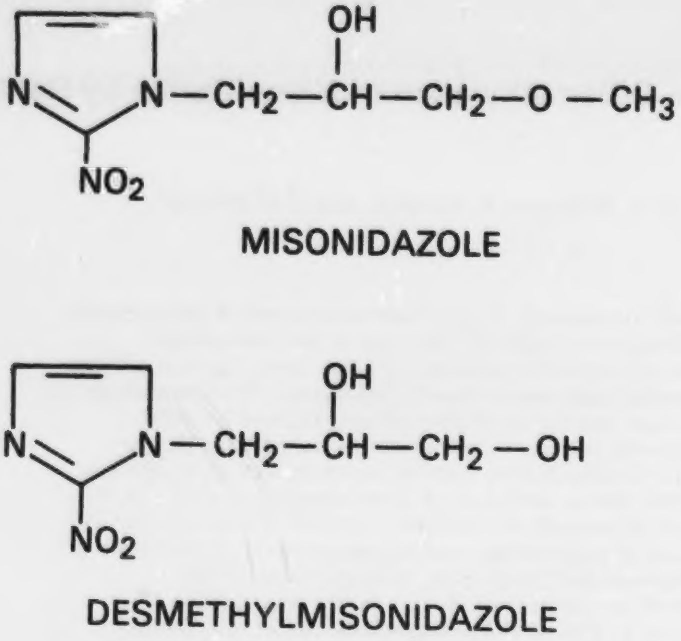

FIGURE 1.—Structures of misonidazole and desmethylmisonidazole.

St. Louis, MO) were killed by decapitation. For the induction studies, rats were pretreated daily for 7 days with PB sodium at a dose of 75 mg/kg ip or saline and were killed by decapitation 24 hours after the last dose. The livers were removed, homogenized in ice-cold 0.1 M sodium phosphate buffer (pH 7.4), and subjected to centrifugal fractionation. The resulting microsomal pellet was washed once and resuspended. The microsomal protein concentrations were determined by the method of Lowry et al (10) using bovine serum albumin as the standard. The formation of desmethylmisonidazole was measured in 0.5 ml of incubation mixtures consisting of 1 mg of both NADH and NADPH, 100 mM sodium phosphate buffer (pH 7.4), 1 mg of microsomal protein, and concentrations of misonidazole ranging from 5×10^{-4} M to 5×10^{-3} M. The reactions were initiated by the addition of misonidazole and were incubated at 37°C in a Dubnoff metabolic shaker. At the appropriate times the reactions were stopped by the addition of 2 ml of ethyl acetate, followed by the addition of 0.85 nmol of the internal standard (1-[2-nitro-1-imidazolyl]-3-fluoro-2-propanol) in water, 50 μl of 0.1 M carbonate buffer (pH 10.0), and 200 μl of distilled water. The samples were mixed and centrifuged at 200 × *g* for 5 minutes in a Beckman model J-6B centrifuge. The resulting top layer was removed and reduced to dryness under nitrogen. The precipitate was reconstituted with 60 μl of 19% methanol in water and analyzed by the high-pressure liquid chromatography method of Workman et al (11).

RESULTS AND DISCUSSION

The results in figure 2 demonstrate that the metabolism of misonidazole to desmethylmisonidazole was linear with respect to microsomal protein concentrations up to 2 mg/ml and to incubation periods up to 10 minutes. The metabolism was optimal in a system composed of microsomes, O_2, and NADPH (table 1). When the incubation mixtures were continuously flushed with N_2, which removed the O_2 required to form the metabolite oxidatively, the conversion of misonidazole to the demethylated metabolite was inhibited by 78%. The use of NADH as the sole electron donor decreased the *O*-demethylase activity by 90% as compared to controls with NADPH alone.

The kinetic parameters for the metabolism of misonidazole were determined after incubation of misonidazole concentrations from 5×10^{-4} M to 5×10^{-3} M with liver microsomes prepared from normal

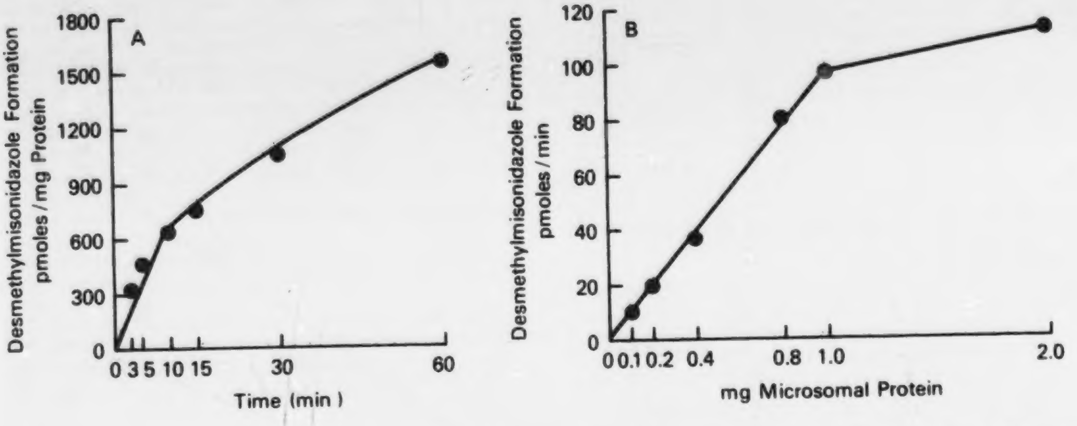

FIGURE 2.—In vitro metabolism of misonidazole to desmethylmisonidazole by rat liver microsomes was measured in 0.5 ml of incubation mixtures. A: formation of desmethylmisonidazole was measured at varying time points as described in the text, and incubation mixtures contained 1 mg of microsomal protein. B: linearity of metabolism was observed after 10 mins of incubation at varying concentrations of rat liver microsomal protein. Values = mean of duplicate determinations of 2 or 3 experiments.

and PB-pretreated rats. The values were fit directly to the following Michaelis-Menten equation:

$$V = \frac{V\max\,[S]}{Km + [S]}$$

using the MLAB program on a PDP-10 computer (12). The apparent Km and Vmax values for normal microsomes were 1.87 ± 0.30 mM and 413 ± 14 pmols/minute/mg of protein, respectively. Pretreatment of rats with PB, a known inducer of hepatic microsomal enzymes (13), prior to preparation of the microsomes resulted in a comparable apparent Km, 1.70 ± 0.52 mM, while the Vmax was increased 2.5-fold to 1033 ± 203 pmols/minute/mg of protein. The Km and Vmax values are the mean ± SE of duplicate determinations of 4–5 experiments. Each incubation mixture of 0.5 ml contained 1 mg of microsomal protein, and the length of incubation was 10 minutes. In figure 3 the actual values are represented by the symbols, while the solid lines are a fit to the MLAB-derived Km and Vmax values.

TABLE 1.—Effect of incubation conditions on the *O*-demethylation of misonidazole*

Incubation condition	*O*-demethylation of misonidazole (pmols/10 mins/mg of protein)†	% inhibition
Complete system	1380.3 ± 64.7	0
+ N_2	304.4 ± 64.7	77.9‡
+ NADH only	145.5 ± 12.3	89.5‡
+ NADPH only	1385.7 ± 54.0	0

*Complete system is described in the text. Flasks for N_2 incubations were flushed continuously.

†Values = mean ± SE of 3 determinations.

‡Significantly different ($P < 0.65$) from value for complete system.

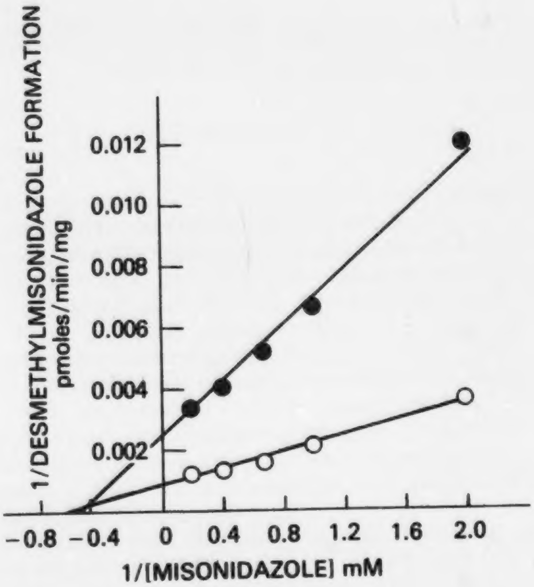

FIGURE 3.—Lineweaver-Burk plot of the *O*-demethylase activity of liver microsomes prepared from normal (●) and PB-pretreated (○) rats. Incubation mixtures contained 1 mg of microsomal protein and totaled 0.5 ml. Duration of incubation was 10 mins. Values = mean of duplicate determinations of 4 or 5 experiments.

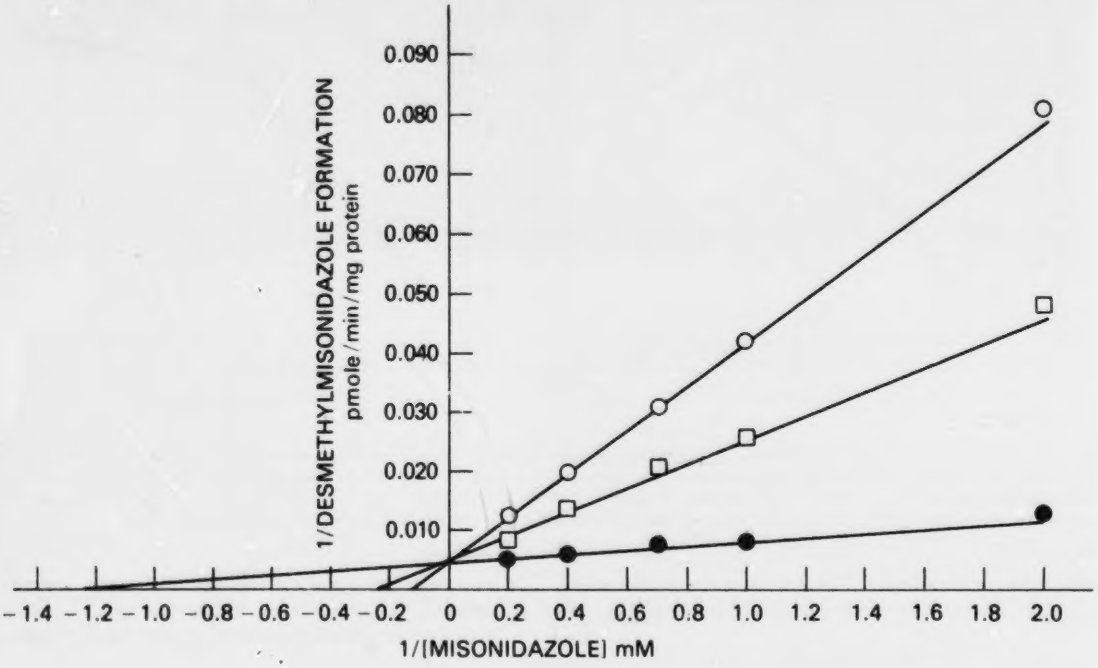

FIGURE 4.—Lineweaver-Burk plot of the inhibition of rat liver microsomal misonidazole *O*-demethylase activity by SKF 525-A. Misonidazole was incubated with 1 mg of rat liver microsomal protein in reaction mixtures totaling 0.5 ml for 10 mins, as described in the text, in the presence of 1×10^{-4} M (○) and 1×10^{-5} M (□) SKF 525-A or the SKF 525-A vehicle (●). Values = mean of duplicate determinations of 2 or 3 experiments.

The observed increase in the Vmax values compares favorably with the increase in the ratio of desmethylmisonidazole to misonidazole observed in rat urine after pretreatment with PB (14). The ratio increased approximately 3.0-fold from 0.8 in saline controls to 2.5 in PB-pretreated rats.

When SKF 525-A, an inhibitor of hepatic microsomal-mediated drug oxidations (15), was added at 10^{-4} and 10^{-5} M to varying concentrations of misonidazole, the resulting inhibition was observed to be of a competitive nature by a plot of the reciprocal values (fig 4). This suggested that misonidazole binds specifically to cytochrome P-450, as SKF 525-A has been shown to interact selectively with cytochrome P-450 (16). The Ki was determined to be approximately 1×10^{-5} M by a direct fit of the data using the MLAB program on a PDP-10 computer to the following equation:

$$V = \frac{\mathrm{Vmax}\,[\mathrm{S}]}{\mathrm{Km}\left(1 + \frac{[\mathrm{I}]}{\mathrm{Ki}}\right) + [\mathrm{S}]}.$$

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Ganglioneuropathy in Rabbits and a Rhesus Monkey Due to High Cumulative Doses of Doxorubicin[1,2]

Roderick T. Bronson,* I. Craig Henderson, and Howard Fixler[3]

SUMMARY

The effects of long-term administration of doxorubicin in adult rabbits and in a rhesus monkey were studied. Eleven rabbits were given cumulative doses of 2–24 mg/kg over a period of 5–28 weeks. The monkey received a total of 20 mg/kg during a 10-month period. One rabbit exhibited hind leg paresis, but all of the other animals in the study remained clinically free of neurologic signs. Rabbits given < 12 mg/kg of doxorubicin had only mild degenerative changes in dorsal roots and a few necrotic neurons in the dorsal root ganglia, but these lesions were much more severe in all rabbits when a cumulative dose > 16 mg/kg was given. The monkey also had severe ganglioneuropathy, suggesting that primates may also be susceptible to this toxic effect of doxorubicin. Although neurotoxicity has not been observed with the dose schedules of doxorubicin used in clinical practice, the tendency of this drug to damage both postmitotic neural and cardiac cells may provide added understanding of the clinically important doxorubicin cardiomyopathy.

[Cancer Treat Rep 66:1349–1355, 1982]

Doxorubicin is both an effective anticancer chemotherapeutic agent and a potent toxin directed against mitotically active normal cells of the gut, hair follicles, and bone marrow (1). Myocardiopathic effects of doxorubicin therapy are well-known in man (2–4) and have been described in experimental animals (5). Paresis associated with necrosis of neurons in the dorsal root ganglia and other peripheral ganglia occurred in rats receiving a single dose of doxorubicin (6,7); no toxic effect on ganglion cells in any other species, including man, has been reported. Although the antimitotic activity of the drug can be explained by its ability to interfere with DNA and RNA synthesis (1), the biochemical basis of its toxic effects on postmitotic myocardial, cardiac ganglionic (8), and peripheral ganglionic cells is not understood.

This study was designed to determine if doxorubicin has neurotoxic effects when given in multiple doses to rabbits and to one rhesus monkey (*Macaca mulatta*). Evidence will be presented that both species develop ganglioneuropathy, the severity of which is dependent on the cumulative dose. Surprisingly, the lesions, although severe in animals receiving high cumulative doses, were clinically obvious in only one rabbit. The dose regimen and total cumulative dose received by the rabbits were similar to those used in rabbits in other experiments (5). They were the maximum tolerable doses that do not cause fatal myelosuppression. In that sense alone, they were somewhat comparable to those used in patients. The findings suggest that some patients treated with doxorubicin might also have subclinical neurohistologic changes.

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[1] Received July 10, 1981; revised Jan 13, 1982; accepted Jan 26, 1982.

[2] Supported in part by Public Health Service grants RR-00168 from the Division of Research Resources and GM-05264, National Research Service Award, from the National Institute of General Medical Sciences, National Institutes of Health, Department of Health and Human Services.

[3] Division of Comparative Pathology, Harvard Medical School, Southborough, MA; Sidney Farber Cancer Institute, Boston, MA; and University of Massachusetts Medical School, Worcester.

*_Reprint requests to:_ Roderick T. Bronson, DVM, Tufts School of Veterinary Medicine, Veterinary Diagnostic Laboratory, 305 South St, Jamaica Plain, MA 02130.

MATERIALS AND METHODS

The rabbits used in these experiments were male New Zealand White rabbits acquired from the Gloucester Rabbitry, Chepachet, RI. They weighed 2.0–2.9 kg at the start of the experiment. The animals were housed in separate stainless steel cages throughout the experiment and were fed 4–8 oz of Charles River Rabbit Formula each day. All animals were inspected daily; hematologic parameters were monitored weekly by cbc.

Eleven rabbits were given weekly injections of 2 mg/kg of doxorubicin administered by rapid iv push through a #22 scalp vein needle placed in the marginal vein of the ear. The cumulative dose varied from 2 to 24 mg/kg. Animals received the treatment until the designated cumulative dose was reached. This required > 12 weeks for animals receiving the highest dose because occasional weekly courses were omitted when the wbc count decreased to < 6000/mm^3 or the platelet count decreased to < 100,000/mm^3. Animals in good health at the time of the last course were permitted to live for 1 month afterwards; those in poor health were killed before that time. One high-dose rabbit was permitted to live for 14 weeks after the last dose to see if long-term sequelae would occur. One control rabbit was killed on the first day of the experiment. Two other controls received eight weekly courses of saline or saline plus dextrose, respectively.

At the conclusion of the experiment, all rabbits were anesthetized with pentobarbital sodium and killed by intracardiac perfusion of a mixture of 4% buffered formaldehyde and 1% glutaraldehyde (9). The central and peripheral nervous tissues were partially dissected and fixation was continued by immersion in the same fixative. One rabbit that developed posterior paresis was killed when moribund; tissues were fixed by immersion in 10% Formalin. For all rabbits, the brain, one trigeminal ganglion, spinal cord, and one or two lumbar dorsal root ganglia with associated dorsal root, ventral roots, and peripheral nerve were sampled and embedded in paraffin. Sections were stained with hematoxylin and eosin (H and E). To compare severity of lesions of different animals, replicate paraffin sections (6-μm thick) of lumbar dorsal roots, dorsal root ganglia, dorsal roots, ventral roots, and peripheral nerves were prepared and stained with H and E, Bodian, and luxol fast blue stains. Small samples of the left myocardium from some rabbits were dehydrated in graded solutions of alcohol and embedded in Epon. Semithick sections were stained with toluidine blue.

Two wild-caught mature female rhesus monkeys were acquired from the New England Regional Primate Research Center. They were housed in standard stainless steel cages and fed commercial chow supplemented with fruits and vegetables. These monkeys were initially included in a study designed to monitor cerebrospinal fluid drug levels, and as part of that study a plastic catheter attached to an Omaya reservoir was placed in the cisterna magna. One monkey was given five courses of doxorubicin at a dose of 4 mg/kg iv, 46, 38, 33, 22, and 12 weeks before death. In addition, the animal received a single dose of 10 g/m^2 of monobutyl methotrexate 72 hours before death. No other drugs were administered. The one other control monkey was given several courses of monobutyl methotrexate but no doxorubicin. The health of the monkeys was monitored by daily inspection and periodic blood cell counts. Post-mortem examinations were performed just after death. Tissues were fixed by immersion in 10% Formalin and processed as described above.

RESULTS

Results of the experiment in rabbits are summarized in table 1. Rabbits 1–9 were in good health throughout the studies. All recovered satisfactorily from marrow suppression after each course of doxorubicin. Of the five rabbits that received larger cumulative doses, one remained in excellent clinical condition throughout the 14 weeks following cessation of treatment and was electively killed. Three others were killed while still receiving weekly injections because they were anorectic and listless. Rabbit 13 was killed when moribund. This animal had been unable to adduct its hind legs and therefore had been unable to stand for 1 week before death. However, it was the only rabbit to develop neurologic signs.

For most of the experimental period the monkey was thin but maintained her appetite. The animal's head tilted slightly to the left following catheter placement in the cisterna magna, but no paresis, paralysis, or other neurologic abnormalities were noted. She was not specifically examined for more subtle neurologic deficits. The monobutyl methotrexate administered 72 hours before death precipitated acute renal failure and heart failure. BUN increased from 19 to 82 mg/100 ml, creatinine increased from 0.6 to 4.8 mg/100 ml, and acute-onset edema and ascites were apparent.

At necropsy, rabbits given lower doses of doxorubicin had no gross lesions. Those given cumulative doses of 24 mg/kg had hydropericardium and dilated hearts. The monkey given doxorubicin had extensive serous effusions in the body cavities and edema of extremities.

Microscopic examination was restricted to the nervous system and myocardium in the rabbits. The

TABLE 1.—Dose regimens used in the study of doxorubicin-induced ganglioneuropathy in rabbits

Rabbit No.	No. of wks of therapy (2 mg/kg)*	Time elapsed between last dose and death (wks)	Cumulative dose (mg/kg)	Severity of dorsal root lesions†	Severity of myocardial lesions‡
1§	0	—	—	0	0
2	1	4.5	2	1+	NT
3	2	4.5	4	1+	NT
4	3	4.5	6	2+	0
5	4	4	8	2+	1+
6	6	4	12	2+	NT
7	8	4	16	3+	2+
8¶	8	4	—	0	0
9**	8	4	—	0	0
10	11	0	20	3+	NT
11	14	0	24	3+	3+
12	13	2	24	3+	3+
13	16	1	24	3+	1+††
14	13	15	24	3+	3+††

*Some weekly doses were omitted because of myelosuppression.

†0 = no lesions; 1+ = occasional vacuoles/several 10× fields; 2+ = a few vacuoles/field; and 3+ = many vacuoles/field.

‡0 = no lesions; 1+ = rare vacuolated myocardial fiber (vf)/several fields; 2+ = 1 vf/high power field (hpf); and 3+ = many vf/hpf. NT = not tested.

§Untreated control.

¶Saline control.

**Saline and dextrose control.

††Scored independently by Billingham et al (2).

nature and severity of lesions varied considerably from rabbit to rabbit (table 1). Control rabbits had no lesions in the peripheral or central nervous systems (fig 1). In rabbits that were killed 1 month after 2–4 mg/kg of doxorubicin, the dorsal roots were normal except for occasional vacuoles containing myelin and axonal debris and lipid-laden macrophages (fig 2). All other portions of the central and peripheral nervous systems were normal. The numbers of vacuoles increased somewhat in rabbits given 6–12 mg/kg, but no more than a few per field of the 10× objective microscopic lens were present. The dorsal root ganglia were generally normal in these, but occasional clusters of satellite cells or neuronophages presumably marked the sites of necrotic ganglion cells.

The rabbits that received cumulative doses ≥ 16 mg/kg had many more vacuoles in the dorsal roots than did the rabbits receiving smaller doses (fig 3). The dorsal root ganglia also had many more clusters of neuronophages (fig 4). Rare neurons had such abnormal features as pyknotic nuclei, cytoplasmic vacuoles (fig 5), and amorphous pale eosinophilic cytoplasmic inclusions (fig 6). The degree of severity of lesions varied in roots and ganglia of rabbits with higher doses. However, at doses > 16 mg/kg no clear-cut dose-response relationship was evident. Whether this reflected variable response on the part of the individual rabbit or of the roots and ganglia was not fully studied here. However, two lumbar ganglia dissected from the rabbit that received a cumulative dose of 12 mg/kg were virtually identical in severity. This was also true for one of the rabbits that received 24 mg/kg.

All ventral roots were completely normal (fig 7). Peripheral nerves distal to the dorsal root ganglia had a few vacuoles. The sciatic nerves studied had no lesions visible by light microscopy. The spinal cords from rabbits with > 16 mg/kg of doxorubicin had vacuoles with shrunken macrophages in the dorsal column and less often in the lateral columns. Ventral horn cells were normal. Trigeminal ganglia examined were similar to the dorsal root ganglia. Vacuoles with gitter cells were present in the spinal trigeminal tract of rabbits that received the larger doses.

The myocardia from the three control rabbits and from the rabbit that received 6 mg/kg of doxorubicin were histologically normal (table 1). By searching many hpfs of the sections from the rabbit that received 8 mg/kg, a rare vacuolated myocardial fiber was found. One such fiber per hpf was present in the section from the rabbit that received 16 mg/kg. Many fibers were vacuolated in three of the four rabbit hearts receiving cumulative doses > 20 mg/kg. Three of the six rabbits with severe neural damage also had severe cardiac damage. In contrast, two of the five rabbits with milder neural damage

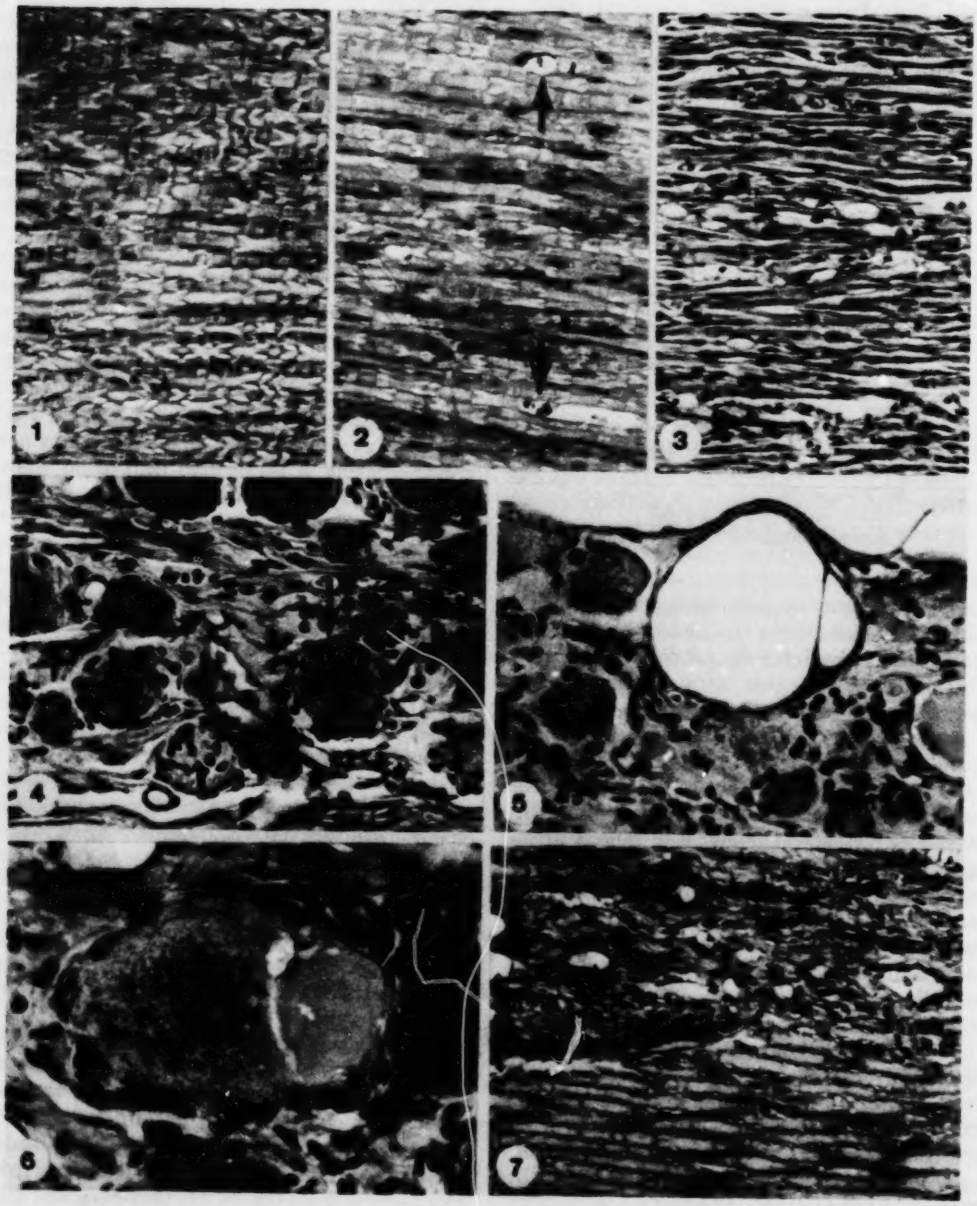
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FIGURE 1.—Longitudinal section of lumbar dorsal root of rabbit 8 that received only saline injection (H and E stain, × 135).

FIGURE 2.—Dorsal root from rabbit 3 that received 2 courses of doxorubicin. Few vacuoles containing pyknotic macrophages and cell debris are present (arrows) (H and E stain, × 135).

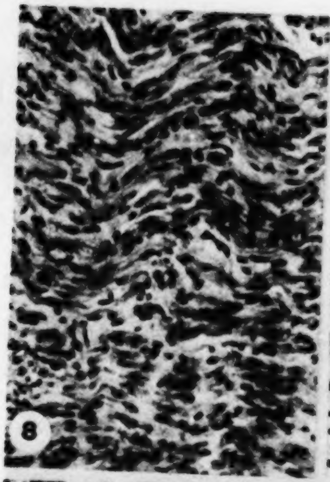
FIGURE 3.—Dorsal root from rabbit 7 that received 8 courses of doxorubicin. Numerous vacuoles are present (H and E stain, × 135).

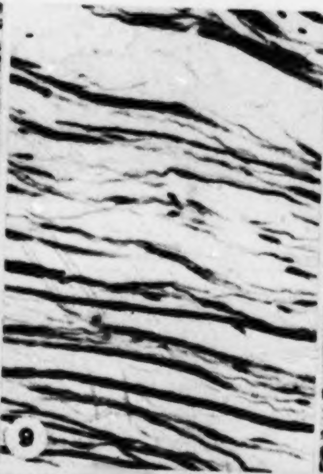
FIGURE 4.—Dorsal root ganglion from rabbit 7. Several ganglionic cells are undergoing neuronophagia (arrows) (H and E stain, × 200).

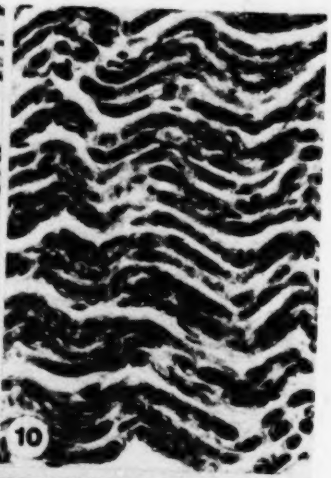
FIGURE 5.—Ganglionic cell from rabbit 13. Cytoplasm is markedly vacuolated and has a peripherally displaced nucleus (H and E stain, × 350).

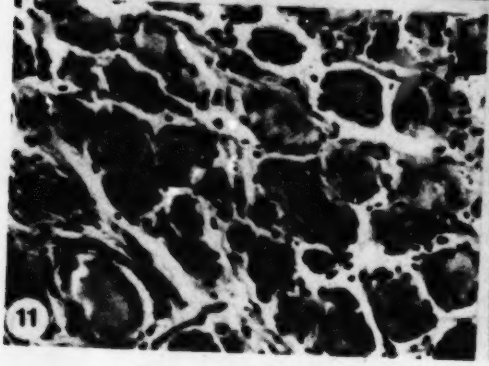
FIGURE 6.—Ganglionic cell from rabbit 10 has a large pale eosinophilic inclusion in the cytoplasm (H and E stain, × 540).

FIGURE 7.—Dorsal root (above) and ventral root (below) of rabbit 13. Dorsal root has many vacuoles. Relative loss of axons and myelin is evidenced by the much greater packing density of Schwann cell nuclei in the dorsal root as compared with the ventral root (H and E stain, × 135).









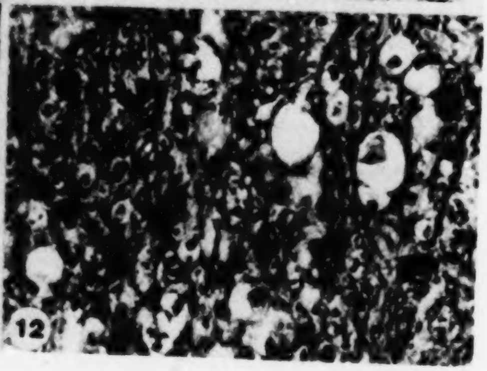


FIGURE 8.—Dorsal root of doxorubicin-treated monkey with loss of axons and myelin (H and E stain, × 135).

FIGURE 9.—Another dorsal root from the same monkey with marked loss of large fibers (H and E stain, × 135).

FIGURE 10.—Ventral root from doxorubicin-treated monkey with normal amounts of myelin and numbers of large myelinated axons (H and E stain, × 135).

FIGURE 11.—Dorsal root ganglion from a monkey that received multiple doses of doxorubicin. Many ganglionic cells are undergoing neuronophagia (H and E stain, × 135).

FIGURE 12.—Cross-section of lumbar spinal cord in the area of the fasciculus gracilis from the doxorubicin-treated monkey. Large vacuoles contain cell debris and macrophages (H and E stain, × 135).

had 0–1 + heart changes; three of these hearts were not examined histologically. Thus, there was a similar dose-toxicity pattern observed in both neural and cardiac tissues.

The monkey given doxorubicin had the most severely affected dorsal roots and ganglia of any animal studied. Some portions of the dorsal roots had numerous vacuoles; others exhibited varying degrees of nerve fiber and myelin loss (figs 8 and 9) as compared to ventral roots (fig 10). Numerous ganglionic cells were replaced by clusters of neuronophages (fig 11). Vacuolated neurons and eosinophilic necrotic neurons were also rarely observed as in the rabbits.

Dorsal columns throughout the spinal cord had numerous vacuoles containing cell debris and macrophages (fig 12). The brain was generally normal. The spinal trigeminal tract on the side contralateral to the catheter was also vacuolated. A glial scar was present in the deep left cerebellar white matter which marked the site of the catheter tip and the fourth ventricle was mildly dilated. There was severe myocardial degeneration and fibrosis in the heart of the monkey. The control monkey given methotrexate alone had no lesions in the brain, spinal cord, dorsal roots, ganglia, peripheral nerve, or myocardium.

DISCUSSION

The neuropathologic observations reported here are not substantially different from those described in doxorubicin-treated rats (6,7). As in rats, unipolar neurons of rabbit and monkey dorsal root ganglia and trigeminal ganglia underwent degeneration. The dose-response, time course, and clinical manifestation in rabbits and in the monkey differed considerably from those in rats, however. In rats, a single dose of 10 mg/kg causes paresis and widespread necrosis in dorsal root ganglia (6,7). In this study, successive weekly doses of 2 mg/kg caused only mild lesions in heart and nervous system at low cumulative doses. At cumulative doses > 16 mg/kg, lesions in both organ systems were more severe. Neurologic signs comparable to the paresis described in rats were present in only one high-dose rabbit. Whether other more subtle abnormalities, such as reduced nerve conduction time or impairment of conscious proprioception, were present was not considered in this study.

In view of the observations in the monkey given doxorubicin it is possible that humans treated with doxorubicin develop a similar ganglioneuropathy. The absence of overt clinical signs in the monkey suggests that such lesions might be clinically silent in man. The issue should be clarified through further experiments that include careful neurologic examination of affected monkeys. At the present time, the clinical significance of these neuropathologic observations in monkeys and rabbits is unknown. The greatest scientific importance of these findings may be in providing further insight into the unusual effects of doxorubicin on the postmitotic cell.

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Altered Hepatic Disposition of Doxorubicin in the Perfused Rat Liver at Hyperthermic Temperatures[1,2]

Joseph L. Skibba,* Frank E. Jones, and Robert E. Condon[3,4]

SUMMARY

The perfused rat liver was used to study the effects of heat on hepatic functions and on the metabolism and biliary excretion of doxorubicin (Ad). Livers were perfused for 1 hour at 37°C and 42°C in the presence of biosynthetic substrates with or without Ad. Gluconeogenesis was decreased at both temperatures in the presence of Ad. Bile flow and excretion of Ad ceased at 42°C. Perfusate levels of Ad were not significantly different in the 1-hour perfusions. Livers were then perfused at 37°C and 41°C for 3 hours in the presence of 10 mM glucose and amino acids. The beta half-life of Ad measured as total fluorescence and as parent drug was prolonged at 41°C. The area under the plasma concentration-time curve was unchanged for parent drug but increased from 2010 μg/ml·min at 37°C to 4477 μg/ml·min at 41°C for total drug equivalents. Biliary excretion of total Ad equivalents was 7.39% ± 1.35% of the total dose at 37°C and 2.56% ± 2.08% of the total dose at 41°C ($P < 0.05$). Biliary excretion of Ad was 2.07% ± 0.37% of the total dose at 37°C and 1.31% ± 0.84% of the total dose at 41°C ($P < 0.05$). Hepatic tissue levels of total Ad equivalents were 288.7 ± 21.2 μg/g at 37°C and 322.7 ± 10.6 μg/g at 41°C ($P < 0.05$). Tissue levels of Ad were 57.5 ± 14 μg/g at 37°C and 23.0 ± 10.7 μg/g at 41°C ($P < 0.05$). Bile flow at the two temperatures decreased after 2 hours of perfusion. The greatest decrease in bile flow occurred during the third hour of perfusion at 41°C. The data indicate that at elevated temperatures there was a significant alteration in the pharmacokinetics of Ad in the perfused rat liver system. Since the liver plays a primary role in the metabolism and elimination of Ad, the observed changes might also occur during whole-body hyperthermia.

[Cancer Treat Rep 66:1357–1363, 1982]

The effects of hyperthermia and of chemotherapy on established tumors and on cells in culture were shown to enhance the cytotoxicity of one another (1). The interaction of doxorubicin (Ad) and hyperthermia has been of particular interest because of the clinical effectiveness of Ad in the treatment of a wide variety of human neoplasms. EMT-6 mammary sarcoma cells (2) and Chinese hamster cells (3) in culture showed increased sensitivity to Ad. Local heating of murine tumors, C3H mammary carcinoma (3), EMT-6 tumor, and KHT mammary carcinoma (4,5) enhanced the cytotoxic effects of systemically administered Ad but failed to enhance the antitumor effects. However, local tumor hyperthermia enhanced the effectiveness of systemically administered Ad against the 16C mouse mammary adenocarcinoma (6). No enhancement of Ad

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[1] Received July 28, 1981; revised Dec 21, 1981; accepted Jan 11, 1982.

[2] Supported by Public Health Service grant CA-27189 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Surgery, The Medical College of Wisconsin, Milwaukee, and Research Service, Veterans Administration Medical Center, Wood, WI.

[4] We thank Joy Sonsalla for her assistance with the liver perfusion system and Bonnie Bates for assistance with preparation of the manuscript.

**Reprint requests to:* Joseph L. Skibba, MD, PhD, Department of Surgery, The Medical College of Wisconsin, 8700 W Wisconsin Ave, Milwaukee, WI 53226.

antitumor activity by whole-body hyperthermia could be demonstrated in the treatment of mice bearing P388 leukemia, mammary adenocarcinoma 16C, and colon carcinoma 38 (7).

There is a paucity of data on the effects of temperature on pharmacokinetics (8). In man, the proportion of salicylamide transformed to its glucuronide was decreased during pyrogen-induced fever (8). Plasma antipyrine half-life was prolonged during etiocholanolone-induced fever, probably due to impaired hepatic metabolism (8). Abnormal sulfobromophthalein retention times were found for all patients with pyrogen-induced fever (8). These results could be the direct effect of heat on hepatic metabolism or changes in renal and hepatic blood flow (9,10). Elevation of body temperature by means of a pump system in animals increased renal blood flow and glomerular filtration rates (9), but temperature increases due to heating the body surface were accompanied by a decline in hepatic and renal blood flow (10).

Hyperthermia significantly prolonged the half-life of antipyrine and the activation of cyclophosphamide in the perfused rat liver (11). A decrease in activity of microsomal suspensions was observed at temperatures > 40°C (12). However, hyperthermia produced minimal effects on the tissue distribution, metabolism, and excretion of Ad in the rabbit (13).

Although Ad may not be of use with whole-body hyperthermia, it could be used systemically with local hyperthermia or in regional perfusion such as in the liver. Therefore, these studies were undertaken to study the effects of hyperthermia and Ad on perfused liver functions and to observe any alterations in the metabolism or disposition of Ad in the isolated perfused liver system at elevated temperatures. Since the liver plays a primary role in the metabolism and elimination of Ad, study of the liver under hyperthermic conditions can also provide results relevant to treatment with whole-body hyperthermia.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley albino rats (King Rat, Madison, WI) weighing 200–250 g were fed laboratory chow ad libitum and fasted for 24–30 hours prior to liver perfusion.

Materials

The enzymes and cofactors necessary for the analysis of perfusate samples were obtained either from Boehringer Mannheim Biochemicals, Indianapolis, IN, or Sigma Chemical Co, St. Louis, MO. Bovine plasma albumin was purchased from Reheis Chemical Co, Phoenix, AZ. All other chemicals were of reagent grade. Ad was from Adria Laboratories Inc, Columbus, OH.

Perfusate

The recirculating perfusate consisted of 150 ml of Krebs-Ringer bicarbonate solution containing 20% (vol/vol) washed outdated human erythrocytes and 2.5% (wt/vol) bovine plasma albumin, Cohn fraction V. During perfusions with an experimental time period of 3 hours, the perfusate also contained 10 mM glucose and amino acids equal to ten times the "normal" concentration of each amino acid in rat plasma. A membrane oxygenator (Sci-Med Life Systems, Inc, Minneapolis, MN) was employed for gassing the perfusate with O_2:CO_2 (95:5).

Perfusion Technique

The technique employed for in situ perfusion of the liver is that described by Collins and Skibba (14) for accurate and precise control of liver temperature. Thermocouple microprobes (Bailey Instrument Co, Saddle Brook, NJ) were used to monitor and control the liver, cabinet, and perfusate temperatures.

A perfusion period of 38 minutes was allowed for equilibration of the preparation. All substrate and drug additions were made immediately thereafter. The livers were perfused for an additional 60–180 minutes after initial sampling at 40 minutes (time zero of the experimental period), allowing 2 minutes for adequate mixing of the drug or substrates.

Analytic Methods

Perfusate samples were obtained at the specified intervals and protein-free filtrates were prepared from the perfusate samples with perchloric acid. Aliquots of protein-free filtrates were analyzed for glucose, lactate, and urea (14). Perfusate O_2 content was determined with Lex-O_2-Con (Lexington Instruments Corp, Waltham, MA). The O_2 content of the inflow cannula perfusate remained constant throughout the experimental period as determined by periodic sampling of the perfusate from the inflow cannula. Perfusate O_2 consumption was determined from the flow rate and difference in O_2 content of the inflow and outflow cannula perfusates.

Ad was determined fluorometrically as total drug equivalents in plasma and bile and as Ad after separation by thin-layer chromatography. Total fluorescence of samples was determined after extraction of 0.5-ml samples of perfusate plasma with 2 ml of ice-cold chloroform:isopropanol (1:1). Total fluorescence of each bile sample was determined after extraction with 10 ml of chloroform:methanol (2:1). Ad was estimated by the methods of Bachur et al (15). The 0.5-ml samples were extracted into chloroform:methanol (2:1), filtered with glass wool, and evaporated to dryness under nitrogen. The residue was redissolved in chloroform:methanol (2:1) and then subjected to ascending single-development chromatography on Silica Gel G thin-layer plates 250 µ (Analtech) in a chloroform:methanol:acetic acid:water (80:20:14:6) solvent system. The fluorescent areas were scraped and eluted with chloroform:methanol (2:1). Fluorescence was determined on an Aminco-Bowman spectrophotofluorometer as Ad equivalents. Activation and emission wavelengths were 485 and 585 nm, respectively. An Ad standard was chromatographed on each thin-layer plate. Recovery of Ad from samples was 80%–90%.

Data Analysis

Estimates of the maximal rates of metabolite change were determined by regression analysis (14). The significance of differences between mean values was determined by Student's t test or analysis of variance. A P value < 0.05 was considered statistically significant. Pharmacokinetic analysis was performed with the aid of a TI Programmable 59 Calculator by linear regression models or by a nonlinear least-squares regression analysis program described by Muir (16).

RESULTS

Effect of Hyperthermia and Ad on Substrate-Stimulated Biosynthesis

Livers were perfused for 1 hour at 37°C and 42°C with an initial perfusate level of 10 µg/ml of Ad. Gluconeogenesis, ureogenesis, and O_2 consumption were measured as indicators of hepatic tolerance. Lactate consumption increased and gluconeogenesis decreased in the presence of Ad at 37°C in comparison to control livers without exposure to Ad (table 1). At 42°C gluconeogenesis was significantly inhibited in the presence of Ad. Overall, the combination of hyperthermia and Ad was minimally detrimental to these hepatic functions. However, bile flow and excretion of Ad ceased at 42°C.

Perfusate samples were analyzed for Ad at intervals after the drug was mixed in the perfusion circuit. The first sample taken after mixing was the zero time for plasma decay analysis. At 37°C the initial Ad level was 2.42 ± 0.42 µg/ml, and this decreased to 0.46 ± 0.24 µg/ml within 60 minutes. At 42°C the initial Ad level was 2.32 ± 0.29 µg/ml, and this decreased to 0.53 ± 0.14 µg/ml within 60 minutes. Samples were measured at 0, 10, 30, and 60 minutes. The data best fit a single exponential curve on linear regression analysis. The correlation coefficients were 0.9. The half-life was 27 minutes at 37°C and 38 minutes at 42°C. The slopes of the decay curves were not significantly different. The hepatic tissue concentration of Ad was 21.38 ± 2.91 µg/g after 1 hour of perfusion at 37°C and 0 µg/g at 42°C.

Effect of Hyperthermia on Disposition of Ad

The 60-minute perfusions were done primarily to study the effects of Ad and temperature on hepatic biosynthetic functions. Ad levels were measured to ensure that each liver was exposed to comparable plasma drug levels. Experiments of longer duration were required to detect any differences in the disposition of Ad at elevated temperatures. In the short experiments, no substrates were added to the perfusate. For longer perfusions, glucose and amino acids were added for maintenance of hepatic functions. Since bile excretion is a primary determinant of Ad disposition and since bile flow ceased at 42°C, a temperature of 41°C was selected to allow study of bile excretion at an elevated temperature. Ad was measured by total fluorescence, which is the total Ad equivalents present including metabolites, and as Ad after separation by thin-layer chromatography. The differences between these two measurements gave an estimate of the amount of Ad converted to metabolites.

After administration of Ad into the reservoir, the perfusate plasma levels declined as shown in figure 1. The time course of the concentration of drug in the reservoir (C) may be described by the biexponential equation

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are the zero-time concentration intercepts associated with the rapid (α) and slow (β) rate constant, respectively. The parameters A, α, B, and β from this two-compartment intravenous pharmacokinetic model were calculated by nonlinear least-squares regression analysis (16). The results are shown in table 2. The half-lives of Ad measured as total fluorescence and as parent drug were prolonged at the higher temperature, 41°C. The area under the plasma concentration-time curve (AUC) was determined on the same program by the linear trapezoidal rule and found to be unchanged for parent drug but increased for total Ad equivalents at

TABLE 1.—Effect of hyperthermia and Ad on hepatic biosynthetic functions

	μmols/g liver/min ($\overline{X}_{max}$ ± SE)*			
Parameter	Control (37°C)	Ad (37°C)	Control (42°C)	Ad (42°C)
Lactate†	−3.21 ± 0.18	−9.16 ± 3.01	−4.65 ± 0.37	−7.85 ± 1.67
Glucose‡	1.36 ± 0.07	0.52 ± 0.09	1.29 ± 0.13	−0.12 ± 0.14
Urea†	1.24 ± 0.10	1.64 ± 0.12	1.01 ± 0.10	1.35 ± 0.25
Oxygen†	4.85 ± 0.22	7.48 ± 0.72	5.79 ± 0.54	7.35 ± 1.20

*Livers from fasted rats were perfused in the presence of 10 mM lactate, 10 mM NH_4Cl, and 2.7 mM ornithine with or without Ad for 1 hr. Perfusate samples were taken every 10 mins for the 60-min perfusion. For each parameter, $\overline{X}_{max}$ refers to maximal rate of change that occurred in 1 of the 10-min periods during 60-min perfusion. Negative values for lactate and glucose indicate consumption. Significance was determined by 2-way analysis of variance.

†Significance level of < 0.05 between corresponding values for control and Ad treatment.

‡Significance level of < 0.05 between corresponding values for control and Ad treatment and between values at the 2 temperatures.

the elevated temperature, 41°C (table 2). The AUC values of 2010 μg/ml·min at 37°C and 4477 μg/ml·min at 41°C indicate marked differences in hepatic clearance of Ad between the two temperatures.

Biliary excretion of Ad was also found to be significantly decreased at 41°C in comparison to that at 37°C (table 3). This includes measurements of total Ad equivalents and parent drug. Hepatic tissue levels of Ad were 57.5 ± 14.0 μg/g at 37°C and 23.0 ± 10.7 μg/g at 41°C ($P < 0.05$). Tissue levels of total Ad equivalents were 288.7 ± 21.2 μg/g at 37°C and 322.7 ± 10.6 μg/g at 41°C ($P < 0.05$).

Bile flow at the two temperatures was not significantly altered until the third 60-minute period of the experiments (table 4).

DISCUSSION

The data demonstrate that Ad and hyperthermia were minimally detrimental to hepatic biosynthetic functions (table 1). Previous results have shown that hepatic functional integrity was maintained from 37°C to 42°C as assessed by gluconeogenesis, urea formation, and oxygen consumption (18). The increase in lactate consumption and decrease in gluconeogenesis at 37°C and 42°C are consistent with the competitive utilization of reducing equivalents for drug metabolism through the malate shuttle as noted by others (19). Gluconeogenesis from lactate and mixed-function oxidation both require oxygen and both share glucose-6-phosphate and malate, either as intermediates in the gluconeogenic sequence or as substrates for NADPH generation.

The alterations in the disposition of Ad produced by hyperthermia in the isolated perfused rat liver are partly in conflict with the results reported for rabbits subjected to whole-body hyperthermia with Ad administration (20). In the rabbits, whole-body hyperthermia was maintained for 1 hour only and the temperatures up to 42.3°C were measured with rectal probes. In this study, no differences were found in the perfusate plasma decay curves of Ad

TABLE 2.—Pharmacokinetic parameters describing distribution of Ad and total Ad equivalents in perfused rat liver at 37°C and 41°C*

	Total fluorescence		Ad	
	37°C	41°C	37°C	41°C
A (μg/ml)	4.61 ± 0.38	4.73 ± 0.27	1.20 ± 0.51	1.45 ± 0.29
α (min $^{-1}$)	0.0429 ± 0.00081	0.0617 ± 0.00894	0.0711 ± 0.7151	0.0430 ± 0.01967
t½, α (min)	16.15 ± (8.4)	11.23 ± (3.58)	9.75 ± (8.7)	16.12 ± (6.2)
B (μg/ml)	1.92 ± 0.45	2.10 ± 0.25	2.10 ± 0.53	0.65 ± 0.35
β (min^{-1})	0.00101 ± 0.00156	0.00048 ± 0.00088	0.0041 ± 0.00212	0.0014 ± 0.00355
t½, β (min)	686 ± (444)	1444 ± (787)†	169 ± (45)	495 ± (235)†
AUC (μg/ml·min)	2010 ± (1270)	4477 ± (2713)†	530 ± (235)	502 ± (240)

*Results were calculated from mean plasma levels. SD values that were determined from analysis of all data sets are listed in parentheses; they are not calculated from the program. Only model macroconstants are listed. Estimation of model macroconstants (A, α, B, β) and their subsequent manipulation to obtain model microconstants (k_{21}, k_{12}, k_{10}) are valid and result in same values as would be obtained by estimating microconstants directly (17). t½ = half-life.

†$P < 0.05$ by t test.

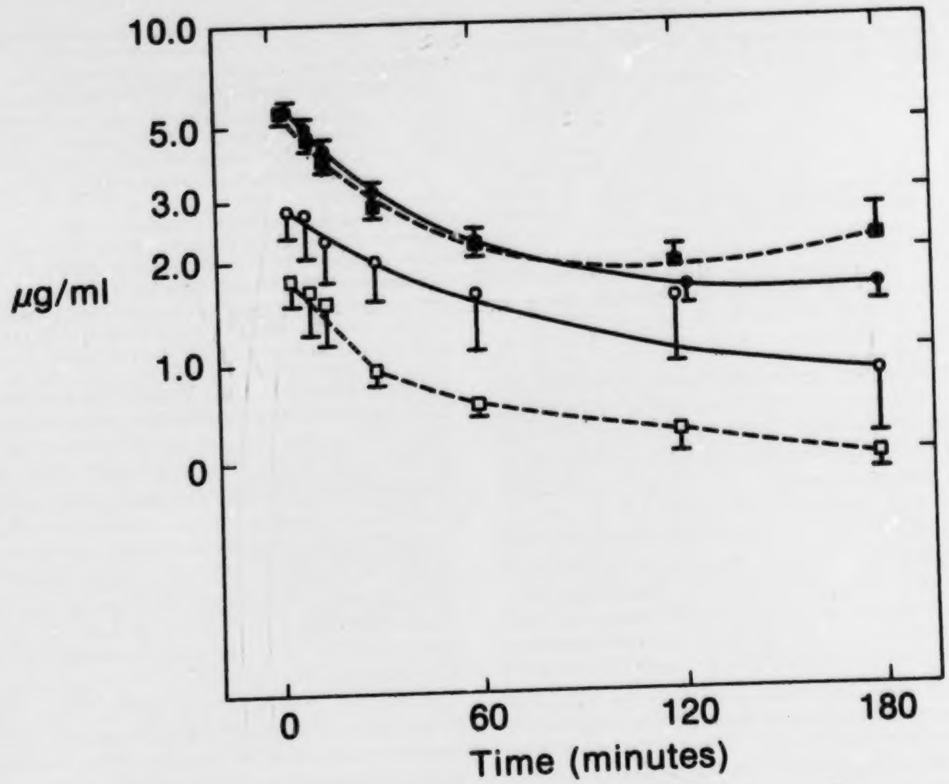

FIGURE 1.—Perfusate plasma concentrations of Ad with an initial calculated level of 15 µg/ml. Samples were obtained at 5, 10, 15, 30, 60, 120, and 180 mins after addition of drug to perfusate reservoir. Data represent mean values of 4–6 perfusions; vertical bars represent SE. ●—● = total fluorescence at 37°C, ■--■ = total fluorescence at 41°C, ○—○ = Ad at 37°C, and □--□ = Ad at 41°C.

at 37°C and 42°C; liver temperature was monitored directly with a needle probe during the 1-hour liver perfusions. One hour may not be long enough to observe significant alterations because of the long plasma half-lives of Ad.

The two-compartment open pharmacokinetic model described the plasma decay of Ad in the whole-animal experiments (13) and in these liver perfusion studies. The beta half-life of the elimination phase of the plasma concentration curve was unchanged in the rabbit by whole-body hyperthermia (13). When the perfusion time was increased to 3 hours, there were significant alterations in the beta half-lives of the elimination phase of Ad and total Ad equivalents when the temperature of the liver was increased from 37°C to 41°C (table 2). The AUC for Ad was unchanged at 41°C from that at 37°C but increased for total Ad equivalents (table 2).

The biliary excretion of Ad plus metabolites was reduced in hyperthermic rabbits to about 50% of control values (13). The biliary excretion of Ad has been studied in bile duct-cannulated rats (20–25). The percent dose of Ad excreted in the bile within 24 hours was 8%–10% (20) and 20% (21). In 3 hours after Ad administration, 25%–27% (23,24) and 4.8% (21) of the dose was found in the bile. The amount of total Ad equivalents excreted in the bile of perfused livers was 7.4% of the administered dose at 37°C (table 3). There was a significant decrease in the biliary excretion of Ad and total equivalents at 41°C.

TABLE 3.—Effect of hyperthermia on biliary excretion of Ad

	% dose excreted in 3 hrs ($\bar{X}$ ± SD)	
Temperature	Total fluorescence	Ad
37°C	7.39 ± 1.35	2.07 ± 0.37
41°C	2.56 ± 2.08*	1.31 ± 0.84*

*$P < 0.05$ by t test, n = 6.

TABLE 4.—Bile flow*

Time (mins)	μl/g liver ± SD			
	37°C		41°C	
	Control (n = 4)	Ad (n = 6)	Control (n = 4)	Ad (n = 6)
0 – 60	26.18 ± 8.29	54.52 ± 19.70	50.18 ± 10.13	43.54 ± 17.41
60 – 120	22.40 ± 6.34	44.36 ± 28.04	26.74 ± 8.52	20.51 ± 19.04
120 – 180	16.92 ± 4.85	29.45 ± 21.75	2.42 ± 0.90	2.38 ± 4.40

*Overall effect of time, ie, a decrease in bile flow, is significant at the 0.05 level. There is a significant (< 0.05) overall time-temperature interaction but no significant effect from Ad. Significance determined by 3-way analysis of variance.

The mechanism for the inhibition of biliary excretion of Ad is unknown. Cholestasis induced by heat could be a cause. When bile flow in rats was inhibited by administration of sodium taurolithocholate, the biliary excretion of total Ad equivalents decreased and the plasma concentrations increased in proportion to the degree of bile flow reduction (24). Although the biliary excretion of Ad and total Ad equivalents was decreased in this study (table 3), the plasma levels were not increased concomitantly. Moreover, the bile flow did not decrease until the third hour of perfusion (table 4). Decrease in bile flow with increasing length of rat liver perfusion is consistent with previous observations by other investigators (25–27). Brauer et al (28) demonstrated that bile production was temperature-dependent in the perfused rat liver; it was maximal between 38°C and 40°C. Both bile flow and sulfobromophthalein transport maximum were increased with a perfusate temperature of 40°C in comparison to values noted at 37°C, but the experiments were performed with the choleretic stimulus of a constant infusion of sodium taurocholate (26). Roberts et al (29) noted that bile flow in rats increased with temperature, but 39°C was the highest temperature studied.

Although bile secretory function appears to be a determinant of Ad disposition at normothermia, other mechanisms are operative at hyperthermic temperatures. The cause of decreased biliary excretion of Ad by hyperthermia could be the result of injury to the cell membrane. At 42°C there was no uptake of Ad into liver tissue. At 41°C the total amount of Ad equivalents in liver tissue was increased over that at 37°C, and the amount of intact Ad in the liver was less at 41°C than at 37°C, suggesting increased metabolism of Ad at 41°C with concomitant decrease in transport from the liver. When bile secretory function was impaired with sodium taurolithocholate, the tissue levels of Ad equivalents increased with increasing cholestasis (24). Thus, transport of Ad from the hepatocyte may be the site of hyperthermic injury. Plasma membrane proteins of several cell types undergo major structural alterations in the physiologic-hyperthermic temperature range [see review by Wallach (30)]. Heat was shown to modify membrane permeability to Ad (2,3).

There are possible clinical consequences from these changes in Ad disposition. Enhanced toxic reactions might be sequelae if Ad was administered in combination with whole-body hyperthermia, since hepatic elimination is a primary determinant of the disposition of Ad. This could perhaps be corrected by reducing the dose of Ad or administering the therapies in a sequential manner rather than concomitantly. There should be no effects on Ad disposition occurring during local hyperthermia treatments unless these treatments are directed to the liver.

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Development and Application of a Radioimmunoassay for Peplomycin[1,2]

G. Wynne Aherne,[3,*] N. Saesow,[3,4] S. James,[3,5] and V. Marks[3]

SUMMARY

A rapid sensitive radioimmunoassay for peplomycin (PM) has been developed using an antiserum produced in a sheep in response to a bovine serum albumin-drug conjugate. Radioiodinated PM was prepared using Iodogen. The assay was reproducible with a limit of detection in plasma of 200 ng/liter. The antiserum does not cross-react with bleomycin or with a number of unrelated drugs. Pharmacokinetic studies using the assay were carried out in three patients who received a short iv infusion of 5 mg/m^2 of PM. The drug disappeared from the plasma with an elimination half-life of approximately 3 hours.

[Cancer Treat Rep 66:1365–1370, 1982]

Peplomycin (PM) is an analog of bleomycin and can be distinguished from bleomycin by the substitution of a 3-[(*S*)-1′-phenylethylamino] propylamino group in the terminal amine position (1). Preclinical studies have indicated that the drug, while retaining the antibiotic and antitumor properties of bleomycin, exhibited less pulmonary toxicity than bleomycin (2,3). Despite decreased lung toxicity, PM was more lethal, with a higher mortality than bleomycin in studies on mice bearing Lewis lung carcinoma and B16 melanoma (4). In the same studies, a peculiar central nervous system toxicity was also noticed with PM.

Radioimmunoassay (RIA) techniques for measuring bleomycin (5–7) and talisomycin (8) in biologic samples have been described and are useful for defining the pharmacokinetic properties of the drug (9–11). We have found that prolonged elevated blood levels of bleomycin in a small number of patients were associated with drug toxicity (unpublished results). It is important that pharmacokinetic studies on PM be carried out during phase II and III trials on the drug, and an RIA suitable for that purpose has been developed and applied to the measurement of plasma drug concentrations in a small number of patients.

MATERIALS AND METHODS

Materials.—PM sulphate (Lot 201) was donated by Lundbeck Ltd, Luton, United Kingdom (UK). Bovine serum albumin (BSA) fraction V, bovine gamma globulin (BGG), ethyl (dimethyl amino propyl)-γ-carbodiimide (EDC), Norit A charcoal, and gelatin were purchased from Sigma Chemicals Co, Ltd, Dorset, UK, Sephadex G10 and Dextran T70 from Pharmacia, Uppsala, Sweden, Iodogen (1,3,4,6-tetra-chloro, 3α-6α-diphenylglycol uril) from Pierce and Warriner, Chester, UK, and Na^{125}I (IMS 30) from Amersham International Ltd, Amersham, UK. Other chemicals were of reagent grade and obtained from BDH Chemicals Ltd, UK. Half lop rabbits were obtained from Ranch Rabbits Ltd, Crawley, UK.

Preparation of immunogen.—PM (45 mg) and BSA (50 mg) were dissolved in 2.5 ml of distilled water,

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[1] Received Aug 31, 1981; revised Dec 7, 1981; accepted Dec 15, 1981.

[2] Supported by the Leukaemia Research Fund, Cancer Research Campaign and Lundbeck Ltd. N. Saesow is the recipient of an International Atomic Energy Authority fellowship.

[3] Division of Clinical Biochemistry, Department of Biochemistry, University of Surrey, Guildford, United Kingdom.

[4] On leave from Nuclear Medicine Section, Department of Radiology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

[5] *Present address:* Department of Pharmacy, Brighton Polytechnic, Moulsecombe, Sussex, United Kingdom.

[*] *Reprint requests to:* G. Wynne Aherne, PhD, Division of Clinical Biochemistry, Department of Biochemistry, University of Surrey, Guildford, Surrey, GU2 5XH, United Kingdom.

EDC (35 mg) was added, and the solution was stirred overnight. In a similar reaction, PM (22.5 mg), BGG (25 mg), and EDC (17.5 mg) were mixed in 1.25 ml of water for 3 hours. Unconjugated PM was removed by dialysis against several changes of 500 ml of distilled water. The amount of unconjugated drug was determined by ultraviolet analysis (285 nm) of the dialysates. The molar incorporation of PM was 9 and 6 for the BSA and BGG conjugates, respectively. The immunogens were stored at 4°C at an approximate protein concentration of 5 g/liter.

Immunization procedures.—One Soay sheep and one rabbit were injected with the PM-BSA conjugate, and two rabbits were injected with the PM-BGG conjugate. The injections were prepared by emulsifying one volume of aqueous conjugate with two volumes of nonulcerative complete Freund's adjuvant (12). The priming injection contained 5 mg of conjugate for the sheep and 2.5 mg of conjugate for the rabbits. The animals were injected in 4–6 intramuscular sites on the legs and back. Booster immunizations were carried out at intervals using half the quantity of immunogen in incomplete adjuvant. At intervals following the injections, blood was collected from the jugular vein of the sheep and the lateral ear veins of the rabbits. The serum was separated and stored at 4°C after the addition of sodium azide at a final concentration of 0.1% (wt/vol).

Preparation of radioiodinated PM.—Iodogen (20 μg) was deposited from dichloromethane (20 μl) onto the bottom of a polypropylene tube (Luckhams Ltd, LP3P). Five micrograms of PM sulphate in 10 μl of 0.2 M borate buffer (pH 8.2) and 0.5 millicurie of Na^{125}I were added to the tube, and the tube was capped and gently vibrated for 2 minutes. A 200-μl volume of 0.1 M phosphate buffer (pH 7.4) was used to transfer the contents of the tube to a Sephadex G10 column (1 × 10 cm), and radioiodinated drug was eluted in 1-ml fractions with assay buffer (*see* assay procedure). The fraction containing the most radioactivity, eluted with 5–6 ml of buffer, was retained and stored at 4°C for use in the RIA.

Assay procedure.—The assay buffer used throughout the procedure (table 1) was 0.05 M phosphate (pH 7.4) containing 1 g/liter of gelatin and 9 g/liter of NaCl. All dilutions and additions were performed using Compupet (Warner Diagnostics Ltd., Eastleigh, UK) or Oxford dispensers. ^{125}I-PM was diluted immediately before use so that approximately 12,000 cpm was added to each tube. The antiserum was diluted so that 50% of the immunoreactive label (maximum bound in the presence of excess antibody) was bound in the zero standard tube. PM sulphate (100 mg/liter) in 0.05 M phosphate buffer (pH 7.4) was stored for up to 2 months at 4°C and was diluted immediately before use as the standard in the assay. Samples were assayed in duplicate at three or four appropriate dilutions. The reagents were added in the order indicated in table 1 to plastic tubes (Luckhams Ltd, LP3), mixed, and incubated at 4°C for 1 hour. Dextran-coated charcoal, prepared as described earlier (13), was added, and after 10 minutes, the tubes were centrifuged, the supernatant was aspirated, and the charcoal pellet was counted.

RESULTS

Antiserum and Label Preparation

PM antisera were obtained following the priming injection from all the immunized animals. Subsequent booster injections increased the antiserum

TABLE 1.—Protocol for RIA of PM

	Volume of reagents added (μl)				
Reagent	Total counts tube	Nonspecific binding tube	Maximum binding tube	Zero tube/ antiserum dilution curve	Standard or sample tube
Assay buffer	—	500	—	400	300
Standard/sample	—	—	—	—	100
Antiserum dilution	—	—	500	100	100
^{125}I-PM	100	100	100	100	100
Mix and incubate at 4°C for 1 hr					
Dextran-coated charcoal	—	100	100	100	100
Mix and incubate at 4°C for 10 mins					
Centrifuge, aspirate supernatant, and count charcoal pellet					

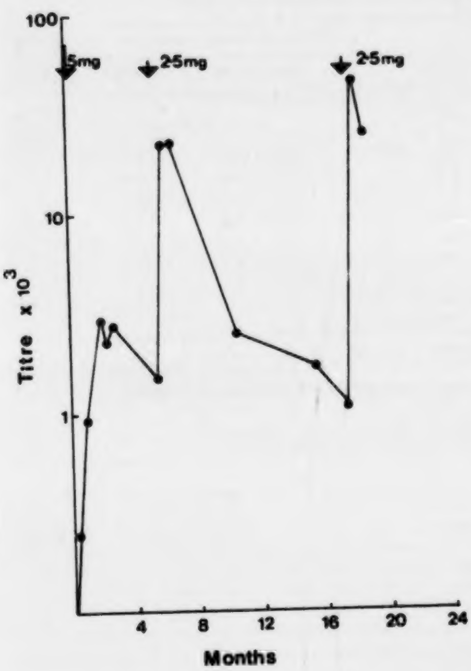

FIGURE 1.—Production of antibodies in response to PM-BSA in sheep G/S/684. Titer was that dilution of antiserum which bound 50% of immunoreactive ^{125}I-PM.

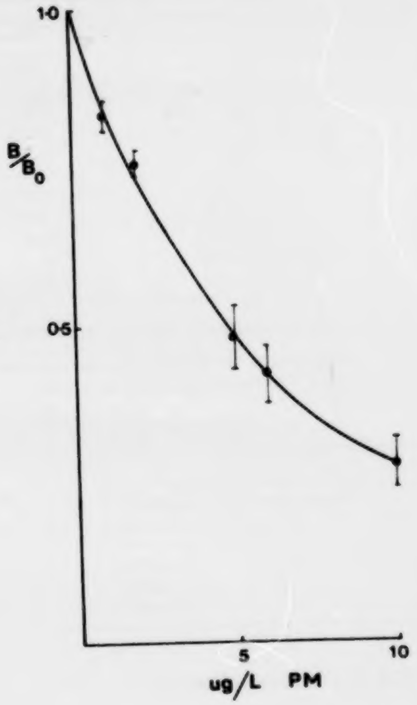

FIGURE 2.—Mean standard curve for PM (n = 10) ± SD. B/B_0 is the ratio of antibody-bound label at standard concentration to antibody-bound label at zero standard concentration.

titers considerably. Preimmunization sera did not bind the iodinated drug. Figure 1 shows the antibody response to immunogen in the sheep G/S/684 immunized with PM-BSA. A pool of antisera collected following the first booster injection (22 weeks) was subsequently used to develop and validate the RIA and could be used at an initial dilution of approximately 1:20,000, depending on the specific activity of the batches of tracer used.

Radioimmunoassay

The displacement of ^{125}I-PM from antibody binding sites by PM is shown in figure 2 and is the mean curve of ten consecutive standard curves. Table 2 shows the within-assay and between-assay variation of the standard curve. Standard curves obtained at 4°C with incubations for 1 and 3 hours, and at room temperature with incubations for 1 hour and 24 hours were identical; for practical reasons it was decided to use an incubation time of 1 hour at 4°C.

Standard curves were constructed with 0, 10, 20, and 50 µl of normal pooled drug-free serum and 0, 5, and 10 µl of normal human pooled urine added. The maximum volume of sample that would be added to the assay is represented by 50 µl (ie, dilution of 1–2) although most samples would be assayed at considerably higher dilutions. There was no effect of either serum or urine on the shape of the standard curve or the percent of binding.

The theoretic limit of detection of the RIA was calculated from the slope of the curve and the standard deviation of the zero point (14) to be 100 ng/liter. The avidity of the antiserum was determined to be 8 × 10^9 L/M using a Scatchard plot (15) and 7.0 × 10^9 L/M using a method described by Muller (16). Bleomycin did not inhibit the binding of ^{125}I-PM to antibody at bleomycin concentrations as high as 100 mg/liter, which represents a cross-reactivity of much less than 0.005% compared with PM. Methotrexate, vincristine, etoposide, cytarabine, 5-FU, and doxorubicin, as well as a number of noncytotoxic drugs, did not cross-react with the antibody at concentrations of 100 mg/liter.

TABLE 2.—Within- and between-batch variation of the PM standard curve (n = 10)*

	Within-batch variation		Between-batch variation	
	Mean ± SD	CV%	Mean ± SD	CV%
Total (T) cpm	11629 ± 64	0.6		
NSB (% T)	0.65 ± 0.47	7.2		
MB (% NSB)	91.1 ± 0.53	0.6	90.9 ± 1.8	2.0
Zero (% NSB)	61.9 ± 1.0	1.6	61.5 ± 4.2	6.8
1 ng/ml (% zero)	79.2 ± 3.75	4.7	83.2 ± 2.7	3.3
2 ng/ml (% zero)	72.9 ± 1.37	1.9	75.8 ± 2.3	3.1
5 ng/ml (% zero)	45.0 ± 3.15	7.0	48.1 ± 5.3	11.0
6 ng/ml (% zero)	38.2 ± 2.39	6.0	42.3 ± 4.6	10.7
10 ng/ml (% zero)	24.5 ± 2.09	8.5	28.2 ± 3.9	13.7

*NSB (nonspecific binding) was calculated as $\frac{\text{total cpm} - \text{NSB cpm}}{\text{total cpm}} \times 100$. MB (maximum binding using excess antibody) and zero standard binding were calculated as a % of the cpm in the NSB tubes. The standard curve was calculated as a % of zero standard binding. CV = coefficient of variation.

TABLE 3.—Recovery of drug from a pool of normal drug-free human serum spiked with PM

Concentration of PM in spiked normal human serum	% recovered ± SD	n	CV%
1 mg/liter	117.4 ± 14.4	10	12.2
100 µg/liter	99.0 ± 11.6	7	11.7
10 µg/liter	97.7 ± 11.5	9	11.8

RIA of PM in Plasma Samples

The recovery of PM added to a normal drug-free plasma pool is shown in table 3. PM concentrations were measured (and the assay repeated over a period of 2 months) in plasma samples obtained from two patients given 5 mg/m² of PM iv over 10 minutes (table 4). The amount of PM bound to plasma proteins was determined in two plasma pools using equilibrium dialysis experiments. Small circular perspex cells (volume, 2 ml) divided by a single layer of Visking tubing were used; 1 ml of plasma was placed on one side of the membrane and 1 ml of 0.1 M phosphate buffer (pH 7.4) was placed on the other side. The cells were closed and rotated overnight at room temperature. The concentration of PM on each side of the dialysis membrane was measured using the RIA. Assuming that equilibrium had been reached and that there had been no binding of drug to the membrane itself, the amount of drug measured on the buffer side of the membrane (the dialysate) was equal to 50% of the free drug, ie, nonprotein-bound drug. The results are shown in table 5.

The disappearance of PM in plasma over 20 hours following the administration of drug (5 mg/m² by 10-minute infusion) was measured in three patients[6] (fig 3). The results are expressed as immunoreactive PM, since the specificity of the antiserum towards metabolites of PM is not known. Results were analyzed according to a two-compartment model following an iv bolus injection. The equation $C_t = A_e^{-\alpha t} + B_e^{-\beta t}$ was calculated for each patient by a nonlinear regression analysis of the data using a BASIC program for a Hewlett-Packard 85A microcomputer. Pharmacokinetic parameters were calculated from the computed values of the constants in the equation and are shown in table 6.

DISCUSSION

A sensitive RIA for PM, which is suitable for pharmacokinetic studies on the drug, has been developed. The antiserum used in the assay was produced in response to a protein conjugate prepared in a manner similar to that described for bleomycin. Specificity studies on bleomycin antisera have shown that alteration of the chemical structure in the nucleus of the molecule, or absence of the terminal side chain, eliminated antibody binding. Chemical modifications in the terminal side chain were recognized by the antisera to various extents and the metabolite desamido-bleomycin cross-reacted significantly (17).

Although specificity studies other than with bleomycin have not yet been carried out, it can be pre-

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[6] Plasma samples were supplied by Dr. P. G. Sorenson, Finsen Institute, Copenhagen, Denmark.

Table 4.—Between-batch variation of the measurement of PM in plasma samples

	Time (mins)	Concentration of PM (μg/liter) ± SD	n	CV%
Patient A	30	422 ± 28.4	5	6.7
	40	663 ± 54.7	5	8.7
	60	363 ± 49.9	6	13.7
Patient B	5	1495		
	20	729		
	40	625		
	60	663		
Pool 1*		825 ± 43.5	10	5.3
Pool 2*		653 ± 32.8	10	5.0

*Samples were randomly pooled from patients A + B for between-batch assays.

Table 5.—Measurement of free and plasma-bound PM by equilibrium dialysis

	PM concentration in g/ml						
Pool	Undialyzed plasma pool	Dialysate (F)	Dialyzed plasma pool (B)	Amount recovered (%)	Free drug (2 × F)	Protein-bound drug (B − F)	% bound
1	851	217	365	582 (68.5)	434	148	25.4
	650	157	368	525 (80.7)	314	211	40.0
2*	607	186	363	549 (90.6)	372	177	32.2

*Protein binding in pool 2 was determined on 2 occasions.

dicted that the PM antiserum used in this assay will display characteristics similar to those of the bleomycin antisera concerning structural changes in the molecule. Thus, because the presence and number of any metabolites of PM and their effect on the binding of the antibodies is presently unknown, results are expressed as "immunoreactive PM." However, the assay is specific as far as a number of unrelated drugs are concerned and will be useful for pharmacokinetic studies in patients who receive a number of cytotoxic and other drugs.

The detection limit of the assay is more than adequate for pharmacokinetic studies and allows the assay to be carried out on low sample volumes. Increased sensitivity where 20 pg of drug can be detected can be achieved using ^{125}I-PM prepared with 1 millicurie of ^{125}I, but the between- and within-batch variations obtained were unsatisfactory. Tracers prepared with 0.5 millicurie of ^{125}I, at the expense of a reduction in sensitivity, resulted in acceptable reproducibility.

The recovery of added drug from serum was complete, although for the 1-mg/liter concentration it was > 100%. The between-batch variation of the assay is good, and it appears that samples can be stored for at least 2 months at 4°C without deterioration.

Preliminary pharmacokinetic studies have indicated that following a short infusion PM disappears from plasma in a biphasic fashion, with an elimination half-life of approximately 3 hours. This figure compared with the half-lives quoted for bleomycin following various routes of administration (18,19).

The dialysis experiment showed that 25%–40% of PM was protein-bound. This is in contrast to results reported for bleomycin (9) where < 1.0% of the drug was protein-bound. The bleomycin experiments were

Table 6.—Computed PM pharmacokinetic parameters

Parameter	Mean value ± SEM (n = 3)
A (μg/ml)	0.056 ± 0.19
B (μg/ml)	0.029 ± 0.03
α (hr^{-1})	0.98 ± 0.18
β (hr^{-1})	0.22 ± 0.01
$t_{1/2}\alpha$ (hr)	0.77 ± 0.17
$t_{1/2}\beta$ (hr)	3.10 ± 0.17
Kel* (hr)	0.46 ± 0.11
V_d area† (liters)	12.1 ± 1.07
Mean plasma clearance (ml/min)	45.0 ± 3.48

*Kel = first-order rate constant for elimination of drug from central compartment.

†V_d = apparent volume of distribution calculated from the area under the curve.

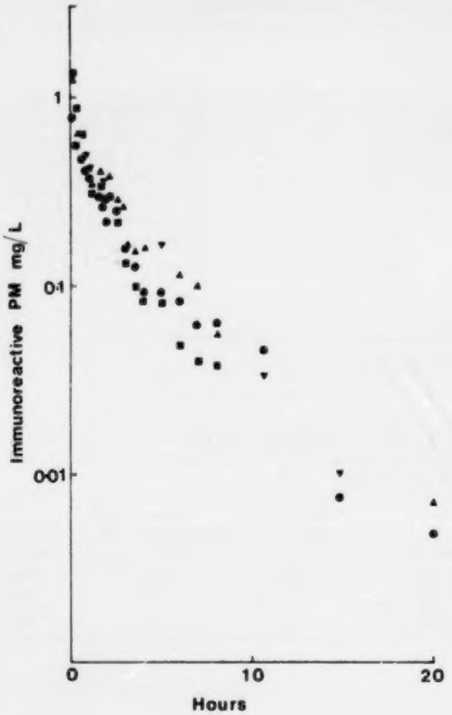

FIGURE 3.—Disappearance of PM from the plasma of 3 patients (● ■ ▼) given 5 mg/m² iv over 10 mins. Blood samples were taken at intervals from the end of the infusion. *See* table 5 for pharmacokinetic data.

carried out after incubation of drug with normal human serum "in vitro," whereas the present study on PM used plasma pools from patients after PM administration. The difference in protein binding of bleomycin and PM may be important in interpreting pharmacokinetic data, and further studies are required to confirm our findings. The availability of an RIA and a recently reported enzyme immunoassay (20) for PM will allow extensive pharmacokinetic studies on the drug in various clinical situations, and these studies may be important in relating toxicity to individual differences in drug elimination.

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Prevention of Doxorubicin-Induced Skin Ulcers in the Rat and Pig With Dimethyl Sulfoxide (DMSO)[1,2,3]

Manubhai H. Desai* and Daniel Teres[4,5]

SUMMARY

Ten potential agents were tested in the Sprague-Dawley rat, using the method of Rudolph et al, which produces skin ulcers with 0.4 mg of doxorubicin intradermally. One antidote, dimethyl sulfoxide (100%, 0.6 ml for 7 days), prevented ulcer formation not only at the local site but also at the doxorubicin control injection site. Similar ulcers were produced in the Yorkshire pig with 2.0 mg of intradermal doxorubicin injection. These ulcers were prevented at both local and distant control sites with dimethyl sulfoxide given for 7 days.

[Cancer Treat Rep 66:1371–1374, 1982]

Doxorubicin, a potent antineoplastic drug, is effective in adult and childhood solid tumors and hematologic cancer. Apart from minor toxic effects such as mucositis, nausea, vomiting, and alopecia, the three major clinical toxic effects are myelosuppression, cumulative dose-related cardiomyopathy, and severe, prolonged skin and subcutaneous ulcers resulting from paravenous drug extravasation.

Cutaneous and subcutaneous ulcerations following paravenous extravasation can be caused by several antineoplastic agents. The possible unique aspects of anthracycline-induced ulcers are their prolonged course with slow healing and their propensity to invade deep structures such as tendons and joints without respect for fascial planes (1).

In 1979, Rudolph et al (2) were able to produce ulcers of predictable size by intradermal rather than sc injection of doxorubicin in laboratory animals. They found that the diameter of the ulcer produced by intradermal doxorubicin injection was related to both the concentration of doxorubicin and the volume of injection. Generally, 0.2 ml of doxorubicin at a concentration of 2 mg/ml produced a 1-cm-diameter lesion that took 7 or 8 weeks to heal.

Apart from measures to prevent paravenous extravasation, many methods of preventing these ulcers have been tried clinically, but the efficacy is uncertain. Suggested methods include subcutaneous injection of sodium bicarbonate or corticosteroids (3) and/or ice pack for 24 hours (4). Excision and grafting have also been recommended. The following study was undertaken to test a large number of potential counteracting agents.

MATERIALS AND METHODS

Sprague-Dawley male rats weighing between 200 and 300 g, with their backs shaved, were used. Doxorubicin and/or antidotes were injected intradermally in the shaved areas. Each rat was kept separately in a cage and was given water and laboratory food ad libitum. The following series of experiments were performed. Doxorubicin (2 mg/ml) dissolved in physiologic saline was used in all experiments.

The first experiment was performed to confirm the results of Rudolph et al (2) and to standardize doxorubicin injections. Three rats in each group were injected intradermally using a 1-ml syringe and a

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[1] Received Sept 14, 1981; revised Dec 21, 1981; accepted Feb 1, 1982.

[2] Supported in part by grants from the Division of Surgery, Baystate Medical Center, Springfield, MA; the Marjorie Sadowsky Memorial Cancer Fund; and the Agricol Corporation, Philadelphia, PA.

[3] Published in part by the American Association for Cancer Research and presented in part at the American Society of Clinical Oncology Annual Meeting, Washington, DC, May 1981.

[4] Baystate Medical Center, Springfield, MA.

[5] We thank Dr. Ronald DeConti for helpful suggestions, Lynn Loring for technical assistance, and Dr. John P. Murnane.

**Reprint requests to:* Manubhai H. Desai, MD, Baystate Medical Center, 759 Chestnut St, Springfield, MA 01107.

25-gauge needle. Physiologic saline and normal saline with 0.9% benzyl alcohol were both used as control injections. The quantity of doxorubicin (2 mg/ml) injected at each site was 0.1, 0.15, 0.2, 0.25, or 0.3 ml. In these rats, weekly measurements of the widest perpendicular diameter of the ulcers were taken with a millimeter ruler. The rats were also photographed weekly and were killed at 8 weeks.

The second experiment screened ten potential agents counteracting doxorubicin skin necrosis in three rats each. The volume of counteracting agent injected at each site was 0.6, 0.3, or 0.15 ml. Physiologic saline and 0.2 ml of doxorubicin were used at control sites. The agents tested were sodium bicarbonate, normal saline, 0.5% benzoic acid, *N*-acetylcysteine, alpha-tocopherol, glutathione, coenzyme Q_{10}, carnitine, methylprednisolone, and dimethyl sulfoxide (DMSO; Sigma Chemical Co, St. Louis, MO). The rats were observed for 4 weeks.

In the third experiment, counteracting agents were screened in three rats immediately after they had each received intradermal injections of doxorubicin at three sites. The volumes of counteracting agents used were 0.6, 0.3, and 0.15 ml. Doxorubicin and saline (both at 0.2 ml) acted as controls in each rat at the fourth and fifth sites. The animals were observed for 4 weeks, with weekly photographs.

In the fourth experiment, DMSO was further tested. Groups of four animals each were injected intradermally with 0.2 ml of doxorubicin. Three animals in each group received DMSO topically (0.6, 0.3, or 0.15 ml) for 7 days. The fourth animal acted as a concurrent control. The animals were observed for 4 weeks.

In the fifth experiment, two groups of three rats each received intradermal doxorubicin (0.2 ml) at two sites each. Each animal in one group was painted with 0.6 ml of DMSO for 7 days at one site only. The second group was painted with 0.6 ml of DMSO at one site for 1 day only. The fourth animal in each group received intradermal doxorubicin (0.2 ml) only.

Because pig skin resembles human skin, the production of doxorubicin-induced skin ulcers was adapted and modified in three 6-week-old Yorkshire pigs. Each pig was injected intradermally with 0.25, 0.5, 0.75, and 1.0 ml of doxorubicin (2 mg/ml) at four different sites. Nine other Yorkshire pigs were injected with 1.0 ml of doxorubicin at two different sites. In three of these pigs, 3 ml of DMSO was painted at one site for 1 day only. In six of these pigs, DMSO was painted daily for 7 days at one site only, with 3 ml of DMSO in three pigs and 1.5 ml in three. The animals were observed for 4–8 weeks, and ulcer diameters were measured and photographs were taken weekly.

RESULTS

We confirm the results of Rudolph et al (2) tha 0.2 ml of doxorubicin (2 mg/ml) produces an ulce approximately 1 × 1 cm. These ulcers develop at week, remain stable for 4–5 weeks, and heal by or 8 weeks. The potential counteracting agents alon did not produce skin ulcers and had no side effect in 4 weeks of observation. When the potential coun teracting agents were injected immediately afte 0.2 ml of doxorubicin, only DMSO prevented ulcers In two animals it also reduced the size of the contro ulcer, which healed quickly, and in one animal, th control ulcer did not develop (table 1). There wa some protection from sodium bicarbonate as well as vitamin E as compared to saline in this screening experiment. The ulcer was smaller with the saline diluent than with any other agent.

In each of six rats, 0.6, 0.3, or 0.15 ml of DMSO was painted daily for 7 days on one site injected intradermally with 0.2 ml of doxorubicin. No ulcers developed in the injection sites painted with 0.6 and 0.3 ml of DMSO. Small ulcers developed in those areas painted with 0.15 ml of DMSO.

In each of six other rats, 0.2 ml of doxorubicin was injected in two separate sites. Three of these rats were painted at one site only for 7 days with 0.6 ml of DMSO. No ulcers developed at either site. Sites on the other three rats were painted on 1 day only. Two rats developed small ulcers at both sites, which healed in 4 weeks. The third rat did not develop any ulcer. Separate concurrent controls developed ulcers 1 × 1 cm.

In three control pigs, 1.0 ml of doxorubicin (2 mg/ml) produced an ulcer approximately 1 × 1 cm at 1 week (fig 1). This ulcer remained stable for 4 weeks. In the three pigs that received DMSO once only at one site, ulcers developed at both control and treated sites at 2 weeks, but the ulcers were approximately one half the size of the ulcers in untreated control animals. Six other pigs that were painted at one site only with DMSO (3 ml in three pigs and 1.5 ml in three pigs) daily for 7 days did not develop ulcers at either site.

DISCUSSION

The aglycone portion of doxorubicin contains a quinone and a hydroquinone ring. Misra and Fridovich (5) have shown that quinone-containing compounds generate active oxygen species during their redox cycle. The formation of superoxide in a liver microsomal system by anthracycline antibiotics was first shown by Handa and Sato (6). Superoxide radical ions can decompose to yield hydroxyradicals,

TABLE 1.—Ulcer diameters at end of Week 1 after injection of doxorubicin* and various doses of potential counteracting agents at different sites

Agent	Rat No.	Ulcer diameter (in cm) following injection with— Saline (0.2 ml)	Doxorubicin	Doxorubicin + 0.6 ml of agent	Doxorubicin + 0.3 ml of agent	Doxorubicin + 0.15 ml of agent
Glutathione (100 mg/ml)	1	0.0	1.0	0.4	0.7	0.5
	2	0.0	1.0	0.6	0.6	0.6
	3	0.0	1.0	0.6	0.7	0.6
DMSO (100%)	1	0.0	0.3	0.0	0.0	0.0
	2	0.0	0.2	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0
L-Carnitine (100 mg/ml)	1	0.0	1.0	0.35	0.45	0.5
	2	0.0	1.0	0.4	0.4	0.5
	3	0.0	1.0	0.4	0.4	0.4
Benzoic acid (0.5%)	1	0.0	1.0	0.5	0.6	0.55
	2	0.0	1.0	0.3	0.4	0.4
	3	0.0	1.0	0.3	0.3	0.5
Alpha-tocopherol (100 units/ml)	1	0.0	1.0	0.2	0.2	0.9
	2	0.0	1.0	0.3	0.3	0.3
	3	0.0	1.0	0.2	0.3	0.2
Sodium bicarbonate (50 mEq/50 ml)	1	0.0	1.0	1.2	0.7	1.0
	2	0.0	1.0	0.2	0.3	0.3
	3	0.0	1.0	0.3	0.2	0.2
Methylprednisolone (40 mg/ml)	1	0.0	1.0	0.7	0.5	0.5
	2	0.0	1.0	0.9	1.0	0.5
	3	0.0	1.0	0.8	1.0	0.5
Coenzyme Q_{10} (5 mg/ml)	1	0.0	1.0	0.8	0.5	0.6
	2	0.0	1.0	0.8	0.5	0.5
	3	0.0	1.0	0.5	0.4	0.6
Bacteriostatic saline with benzyl alcohol (0.9%)	1	0.0	1.0	0.8	0.4	0.5
	2	0.0	1.0	0.8	0.5	0.4
	3	0.0	1.0	0.3	0.3	0.4
N-Acetylcysteine (100 mg/ml)	1	0.0	1.0	0.6	0.5	0.5
	2	0.0	1.0	0.7	0.5	0.4
	3	0.0	1.0	0.5	0.8	0.6

*0.2 ml of doxorubicin (2 mg/ml).

peroxyradicals, and hydrogen peroxide. Such oxygen-free radicals may initiate a peroxidation of endogenous lipids and cause membrane damage (7).

Alpha-tocopherol in DMSO (90%) was shown by Svingen et al (8) to reduce the size of ulcer. DMSO was used as a carrier agent for the relatively insoluble alpha-tocopherol. In their 2-day study, DMSO alone seemed to reduce the size of ulcers both on the treated and untreated flanks of separate controls. Their statistical comparison was made between the control on the opposite flank and not with separate control animals. In our study, DMSO not only prevented ulcer formation at local sites but also at distant sites. Vitamin E alone was not tried by Svingen et al. In our screening experiment with a small number of animals, there was some reduction in the size of ulcers treated with vitamin E. Inability to penetrate and to distribute along tissue planes may be the problem with other free-radical scavengers. There may also be differences in the manufacturing of DMSO or in the concentration or method of application.

DMSO, as a drug solvent that penetrates along all tissue planes, may reduce the concentration of doxorubicin. However, it is unlikely that doxorubicin was extracted out of the skin by DMSO, because DMSO worked as effectively at distal sites. DMSO is a potent hydroxyl radical scavenger (9). If doxorubicin-induced skin ulcers are related to the formation of toxic free radicals of oxygen, then DMSO may prevent ulcers by scavenging of free hydroxyl radicals. Further work is needed in animal models more closely related to human extravasation problems. Also needed is more work in agent combinations, dose- and time-related studies, and pharmacodynamics.

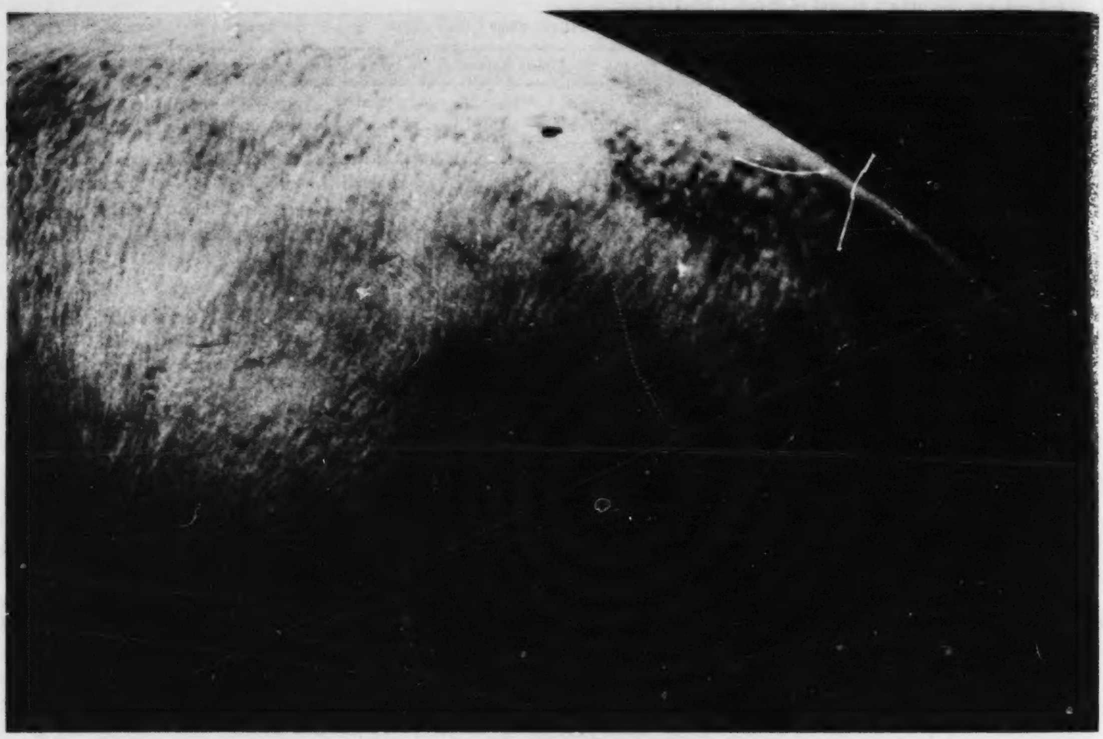

FIGURE 1.—Photograph of a pig showing doxorubicin-induced skin ulcers.

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Prevention and Reversal by a Retinoid of 3,4-Benzpyrene- and Cigarette Smoke Condensate-Induced Hyperplasia and Metaplasia of Rodent Respiratory Epithelia in Organ Culture[1]

Ilse Lasnitzki[2,*] and Werner Bollag[3,4]

SUMMARY

The influence of an aromatic analog of vitamin A, etretinate, on the effects of 3,4-benzpyrene and cigarette smoke condensate has been investigated in fetal mouse lung and neonatal rat tracheas grown in organ culture. In both tissues, 3,4-benzpyrene as well as cigarette smoke condensate induces a striking increase of epithelial mitosis within 12–14 days of treatment. The increase is associated with a loss of secretory activity and of ciliary function. These changes persist in the absence of benzpyrene or smoke condensate in explants transferred to control medium. Treatment with etretinate alone does not affect the normal epithelial growth rate or normal differentiation. If combined with either benzpyrene or smoke condensate, the aromatic compound inhibits the increase in cell division and prevents the loss of secretory activity or ciliary function. In explants pretreated with 3,4-benzpyrene or cigarette smoke condensate, etretinate reduces the carcinogen- or smoke condensate-induced increase in mitotic activity to normal levels and restores secretory differentiation and ciliary function. The mechanism of action involved in the anticarcinogenic activity of the retinoid is discussed.

[Cancer Treat Rep 66:1375–1380, 1982]

Vitamin A is essential for the maintenance of growth, vision, and reproduction and for the differentiation of epithelia. In vitamin A deficiency, epithelia of the respiratory tract and of male accessory sex organs undergo squamous metaplasia. (1–3). Conversely, excess vitamin A induces a mucous metaplasia of squamous keratinizing epithelium (4–7).

There is mounting evidence that, in addition to its physiologic function, vitamin A also possesses anticarcinogenic properties. This was first seen in mouse prostate glands in organ culture in which vitamin A counteracted the effects of the carcinogenic hydrocarbon 3-methylcholanthrene (8,9) and was confirmed in animal experiments. The induction of respiratory tumors in rats and hamsters and that of skin papillomas and carcinomas in mice was inhibited by treatment with vitamin A or vitamin A acid (10–12).

However, the use of vitamin A or vitamin A acid as potential anticancer agents is restricted by their toxicity at pharmacologic concentrations, and in an attempt to reduce the toxicity, a number of structural analogs (retinoids) have been synthesized (13). Among these retinoids is the aromatic retinoid etretinate, in which the cyclohexenyl ring of the ethyl ester of retinoic acid has been replaced by a substituted aromatic ring. This retinoid is highly active in prevention of and therapy for DMBA-induced skin papillomas and carcinomas in mice and possesses a particularly favorable ratio between anti-

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[1] Received Oct 9, 1981; accepted Nov 23, 1981.

[2] Strangeways Research Laboratory, Cambridge, England.

[3] Department of Pharmaceutical Research, F. Hoffmann-La Roche and Co, Ltd, Basel, Switzerland.

[4] We thank Mrs. Dawn Ward and Mrs. Alyson Fisk for their skillful technical assistance.

**Reprint requests to:* Dr. Ilse Lasnitzki, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 4RN, England.

tumor activity and toxic effects (13–15). Furthermore, it reverses methylcholanthrene-induced hyperplasia and metaplasia of prostatic epithelium in vitro (16) and was seen to inhibit the growth of a human bronchial carcinoma implanted into nude mice (17).

The response of secretory epithelia and in particular that of bronchial epithelium to this retinoid prompted the question of whether it may inhibit or delay the early effects of cigarette smoke and its hydrocarbons on respiratory epithelia in general, and it seemed worthwhile to examine the interaction of this potent retinoid and carcinogenic compounds in greater detail and under strictly controlled experimental conditions. The present paper reports experiments in which the influence of etretinate on the effects of 3,4-benzpyrene and cigarette smoke condensate has been investigated in rodent bronchial and tracheal epithelium grown in organ culture. The results may provide valuable clues for clinical trials to assess the influence of etretinate on the progression of precancerous and cancerous conditions of the human respiratory tract.

MATERIALS AND METHODS

The tissues used for the experiments were 17–18-day fetal mouse lungs from a laboratory inbred strain of R mice and tracheas from neonatal rats obtained from a strain of hooded Lister rats. They were grown in organ culture by a modified Trowell technique (18,19). The lungs were divided into fragments measuring approximately 2 × 2 × 3 mm and the tracheas were halved. Six to eight lung fragments and four half-tracheas were explanted on strips of Millipore filters which were placed on grids of stainless steel. The grids rested in small culture chambers filled with medium up to the level of the rafts. The culture chambers were placed in Petri dishes carpeted with moist filter paper to provide a humid atmosphere. The medium used was Parker's 199 (20) supplemented with 15% newborn calf serum which had been irradiated with ultraviolet light to inactivate the retinol present in the serum. Before incubation, the explants were perfused with a mixture of 5% CO_2 and 95% oxygen for 20 minutes at a flow rate of 125 ml/min.

3,4-Benzpyrene or hydrocarbon-enriched neutral fraction of cigarette smoke condensate was added to the medium at concentrations of 5 or 30 µg/ml, respectively. The retinoid etretinate (supplied by F. Hoffmann-La Roche and Co, Ltd) was used at a concentration of 2 µg/ml.

In a first series of experiments, tracheal explants were grown for 14 days with either 3,4-benzpyrene or cigarette smoke condensate alone or combine with the retinoid; lung explants were grown for 1 days with 3,4-benzpyrene or for 12 days with cigarette smoke condensate alone or combined wit the retinoid.

In a second series of experiments, both tissue were first exposed to the carcinogen or cigarett smoke condensate for 12 or 14 days and then transferred to control medium or medium containin etretinate for a further 4 days. To assess the effec of the retinoid alone, additional sets of explants wer treated with etretinate for 12 or 14 days or explant kept previously in control medium were exposed t the retinoid for 4 days.

At the end of the culture period, the explants were incubated with demecolcine (2.0 µg/ml), a metaphase-arresting agent, for 5 hours and then fixed in Bouin's solution, dehydrated, embedded in paraffin, serially sectioned, and stained with hematoxylin-eosin or by the Azan substitute method.

The criteria of effect used were changes in cell differentiation and cell proliferation. The latter was quantitated and expressed as the percent of epithelial mitoses and its standard deviation.

RESULTS

Fetal Mouse Lung

Before explantation, the lungs of 17- or 18-day mouse embryos consist of primitive bronchioli lined with cuboidal epithelium and embedded in cellular connective tissue. Within 12–14 days of growth in control medium, a network of branching bronchi has developed. They are lined with tall secretory cells which show a small number of cell divisions. Addition of the retinoid alone does not affect this normal pattern of growth.

Exposure to 3,4-benzpyrene or smoke condensate increases epithelial mitosis leading to hyperplasia of the bronchial epithelium. In some areas, the hyperplastic epithelium is composed of proliferating basal cells surmounted by the original secretory epithelium, and in others, the superficial secretory cells are shed. In explants pretreated with either benzpyrene or smoke condensate and transferred to control medium, the increase in cell proliferation and hyperplasia persists or progresses in the absence of the carcinogens, and in most hyperplastic foci secretory cells are now absent.

Addition of the retinoid inhibits the induction of hyperplasia and maintains secretory activity if combined with the carcinogens; if administered afterwards, the retinoid reverses the hyperplasia and restores secretory activity.

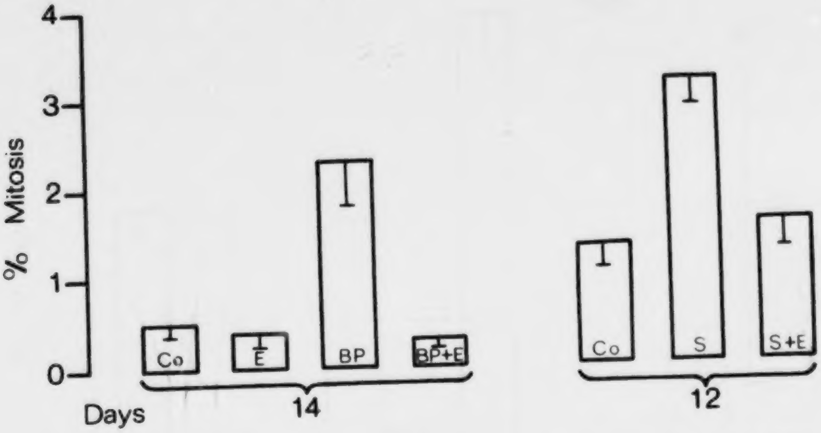

FIGURE 1.—Prevention of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of bronchial epithelium by etretinate (E). Co = control medium.

The mitotic counts support these histologic observations; they are summarized in figures 1 and 2. Treatment with 3,4-benzpyrene raises the incidence of mitosis to over four times the control value, and the cigarette smoke condensate increases it more than twofold. The retinoid alone does not affect the normal mitotic rate but it does prevent the carcinogen- and smoke condensate-induced increase during combined treatment.

In explants pretreated with 3,4-benzpyrene and transferred to control medium, the incidence of mitosis is nearly six times that of the control value and more than threefold in those previously exposed to the smoke condensate. Application of the retinoid alone for 4 days does not alter the normal incidence of mitosis but it reverses the augmentation induced by the carcinogen or smoke condensate.

Rat Trachea

In neonatal rats, the trachea is lined with one row of cuboidal, occasionally ciliated cells, separated from the surrounding ring of cartilage by a thin layer of connective tissue. After 12 or 14 days in control medium, the epithelium has become columnar due to an enlargement of the supranuclear cytoplasmic

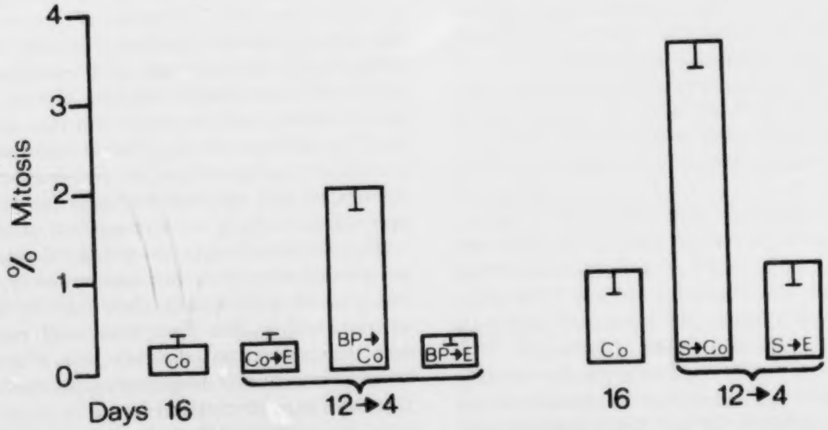

FIGURE 2.—Reversal of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of bronchial epithelium by etretinate (E). Co = control medium.

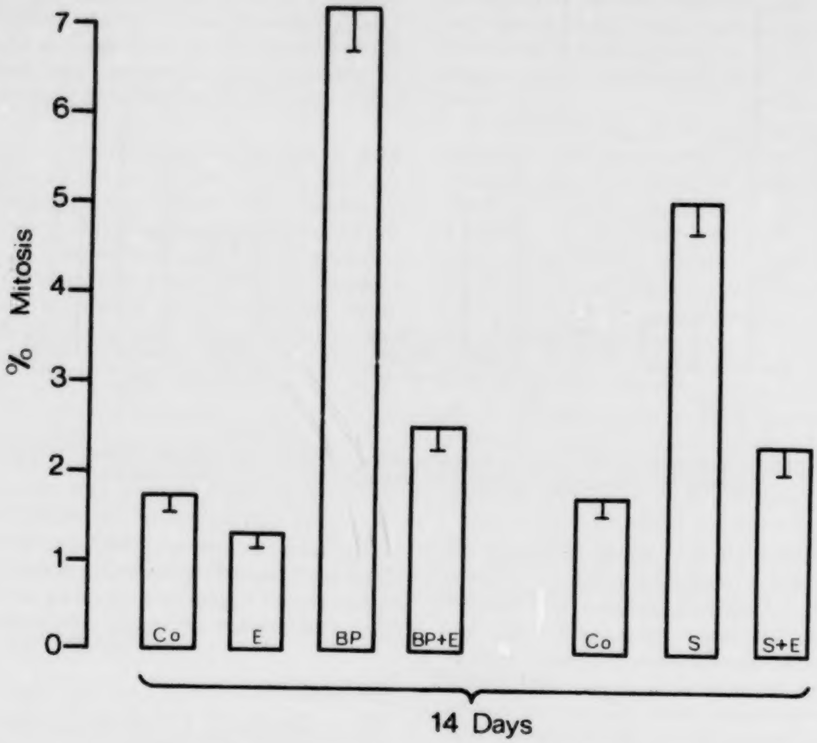

FIGURE 3.—Prevention of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of tracheal epithelium by etretinate (E). Co = control medium.

area and has developed a ciliary apparatus and formed mucin-secreting goblet cells. Occasionally, reserve cells can be seen between the superficial ciliated epithelium and the basement membrane.

Exposure to the aromatic retinoid etretinate does not alter this basic pattern, and the epithelium is indistinguishable from that of the control explants.

In contrast, 3,4-benzpyrene has a profound effect. It stimulates the proliferation of the epithelium, often resulting in a multilayered, often-folded epithelium. At the same time, it has greatly reduced the number of goblet cells and has a deleterious effect on the cilia which have become clumped. In some tracheas, the epithelium shows squamous metaplasia with the formation of strands of keratin.

In explants exposed to cigarette smoke condensate, a similar although somewhat weaker effect can be observed: stimulation of cell proliferation associated with a loss of goblet cells and clumping of cilia.

As in the lung, the changes persist after transfer to control medium. Addition of the retinoid inhibits the benzpyrene- or smoke condensate-induced changes in a striking manner. If combined with either compound, etretinate inhibits the increase in cell proliferation and prevents the loss of goblet cells and the damage to the cilia; if applied after them, it reduces cell proliferation and restores the ciliary apparatus and the formation of goblet cells. Such epithelium closely resembles that of the controls.

The mitotic counts show (figs 3 and 4) that the percent of mitosis is increased over fourfold by 3,4-benzpyrene and nearly threefold by the cigarette smoke condensate. The retinoid alone does not affect the normal mitotic rate, but if combined with benzpyrene or the smoke condensate it reduces the increase to near control levels.

In explants pretreated with either carcinogen or smoke condensate and transferred to control medium, the rate of cell division amounts to six and

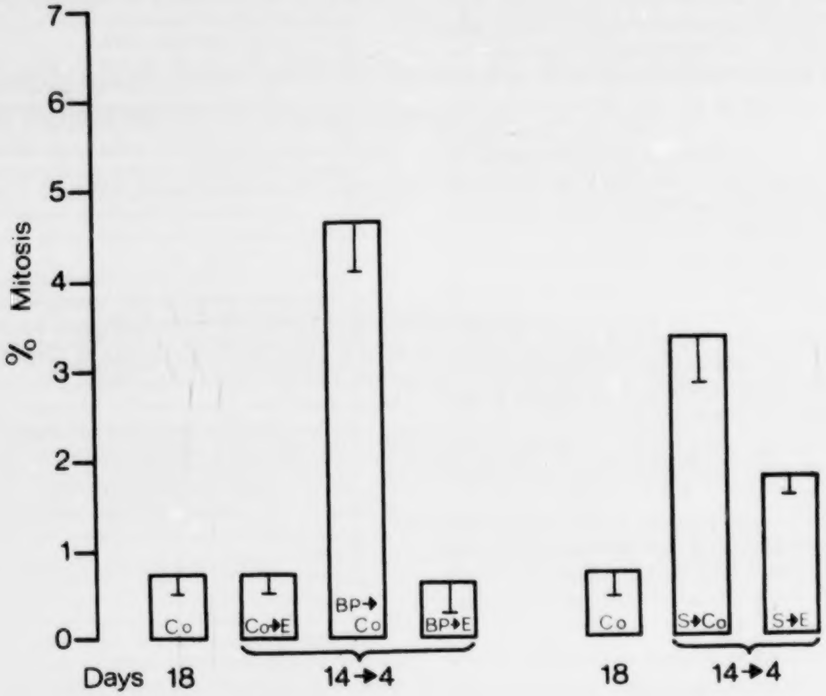

FIGURE 4.—Reversal of benzpyrene (BP)- and cigarette smoke condensate (S)-induced mitotic stimulation of tracheal epithelium by etretinate (E). Co = control medium.

four times the control value, respectively. In both sets, administration of etretinate for 4 days reverses the increase and reduces it significantly.

DISCUSSION

In both fetal mouse lung and neonatal rat trachea, 3,4-benzpyrene and cigarette smoke condensate stimulate epithelial cell proliferation and increase its mitotic rate. The activity of the smoke condensate which contains small quantities of benzpyrene and other carcinogenic and noncarcinogenic hydrocarbons is somewhat lower than that of benzpyrene. In both organs, the increase of epithelial cell replication is accompanied by a loss of secretory activity. In the bronchial epithelium, hyperplastic foci are characterized by crowded basal cells with the loss of the superficial secretory cells; in the trachea, the number of goblet cells is greatly reduced and the cilia are severely damaged. Similar epithelial growth promotion associated with metaplastic changes has been described for benzpyrene- and smoke condensate-treated human fetal lung and mouse tracheal epithelium in vitro (21–23) and for hamster tracheal cultures exposed to benzpyrene (24,25).

Administration of the aromatic retinoid etretinate has a profound effect both on cell replication and differentiation. If combined with benzpyrene or smoke condensate, it inhibits the increase in cell proliferation, maintains the secretory activity of the bronchial epithelium, and prevents the loss of goblet cells and cilia in the trachea. If added following exposure to the carcinogens, it reverses epithelial hyperplasia and restores the secretory activity of the bronchial epithelium as well as goblet cell and cilia formation.

Under both conditions, the action of the retinoid results in architecture and cytology which closely approximate those of the control tissues.

Similar effects of natural and synthetic vitamin A compounds have been reported for the same system. In hamster trachea grown in organ culture, vitamin A inhibited or reversed benzpyrene-induced hyperplasia and metaplasia (24,25), and in vitamin A-deficient tracheal cultures retinoids prevented squamous metaplasia (26).

The mechanism of action involved in the anticarcinogenic activity of the retinoid is not yet fully understood. It is now recognized that retinoids play an important role in the synthesis of glycosaminoglycans and are implicated in the transfer of sugar moieties to glycoproteins (27,28). In both lung and trachea, the restoration of the secretory function is associated with an inhibition of the carcinogen-induced cell replication. It is possible that these two events are linked and that a diminished proliferation rate allows the specialized cell functions to be reestablished. A similar inverse relationship between epithelial DNA synthesis and the reversal of squamous metaplasia was seen in prostatic epithelium exposed to methylcholanthrene and retinoids (29).

However, the link between cell proliferation and differentiation found in secretory epithelia is not seen in all tissues. In DMBA-induced skin papillomas treated with the same aromatic retinoid, the regression is not associated with diminished basal cell replication (30) but can be attributed to the deposition of glycosaminoglycans which disrupt the continuity of the hyperplastic keratinized cell layers and cause their breakdown (31,32). Thus, the mechanisms by which retinoids affect target organs appear to be specific for each given tissue.

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Tubulin-Dependent Biochemical Assay for the Antineoplastic Agent Taxol and Application to Measurement of the Drug in Serum[1]

Ernest Hamel,* Chii M. Lin, and David G. Johns[2,3]

SUMMARY

A biochemical assay for taxol with sensitivity to 0.1 μM has been developed. Taxol-dependent formation of tubulin polymers occurs at 37°C in 1.0 M glutamate in the absence of GTP. These polymers are cold-stable and hydrolyze GTP at 0°C, whereas tubulin alone will not hydrolyze the nucleotide in the cold. This assay has been used to follow rabbit serum levels of taxol injected iv. Although the drug appears to be almost totally protein-bound, it is nevertheless rapidly cleared from serum. The apparent α-phase and β-phase half-lives after iv bolus administration in one rabbit are 2.7 and 42 mins, respectively.

[Cancer Treat Rep 66:1381–1386, 1982]

Taxol, a natural product derived from *Taxus brevifolia* (1), is a promising new antineoplastic agent that soon should enter clinical trials. The target of the drug appears to be the microtubule, but taxol has a unique mechanism of action. Rather than inhibiting tubulin polymerization, it markedly enhances the reaction (2–5). Moreover, microtubules formed in the presence of taxol are unusually stable: they remain intact at low temperatures, in the presence of Ca^{2+}, and after dilution, conditions that normally lead to the rapid disaggregation of microtubules (2–5).

The effect of taxol on tubulin-dependent GTP hydrolysis is highly dependent on reaction conditions (4,5). In some cases no substantial effect on the reaction is observed (4,5), but under other conditions GTP hydrolysis is dependent on the presence of taxol (5). This taxol-dependent GTP hydrolysis is associated with tubulin polymerization, with the drug inducing both polymerization and nucleotide hydrolysis under conditions where they otherwise do not occur (5). In preliminary studies we had found that micromolar taxol concentrations, substoichiometric to the tubulin concentration we were using, resulted in definite stimulation of GTP hydrolysis. Since this approached anticipated serum concentrations of the drug (M. C. Lowe, personal communication), we decided to explore its stimulation of tubulin-dependent GTP hydrolysis as a potential assay for taxol.

MATERIALS AND METHODS

Highly purified calf brain tubulin was prepared as described previously (5,6), including gel filtration chromatography to remove unbound nucleotide. Taxol was obtained from the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute, and was dissolved in dimethylsulfoxide. Nonradioactive GTP from Sigma Chemical Co was repurified by triethylammonium bicarbonate gradient chromatography on DEAE-Sephadex A-25. Stock solutions of monosodium glutamate, purchased from Grand Island Biological Co, were adjusted to pH 6.6 with HCl. Polyethylenimine cellulose thin-layer sheets were from Brinkmann. High specific activity [α-^{32}P]GTP (initially

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[1] Received Jan 20, 1982; accepted Feb 2, 1982.

[2] Laboratory of Medicinal Chemistry and Biology, Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health, Bethesda, MD.

[3] We thank Dr. J. Kelley for assistance with pharmacokinetic calculations, Mr. R. Hyman and Mr. E. Owens for assistance in the animal study, and Ms. B. Cohen for her help in preparing the manuscript.

* *Reprint requests to:* Dr. Ernest Hamel, Laboratory of Medicinal Chemistry and Biology, Developmental Therapeutics Program, National Cancer Institute, Bldg 37, Rm 6D28, Bethesda, MD 20205.

2000–3000 curies/mmol) was purchased from Amersham.

GTP hydrolysis was determined by following the formation of [α-^{32}P]GDP from [α-^{32}P]GTP by thin-layer chromatography in 1.0 M KH_2PO_4 and autoradiography (6). Specific conditions are described in the individual experiments; all assay mixtures contained 10% dimethylsulfoxide. All assays were performed in triplicate. Reactions were stopped by adding 20 μl of reaction mixture to 40 μl of 25% acetic acid. Of this mixture, 10 μl was spotted in triplicate on polyethylenimine cellulose thin-layer chromatography sheets (all experimental points are thus the average of nine values), and chromatography was begun while the spots were still wet.

TABLE 1.—Effect of preincubating taxol and tubulin at 37°C on subsequent GTP hydrolysis at 0°C*

	% GTP hydrolyzed	
	Not preincubated	Preincubated
No taxol	3.7	3.6
+ 1 μM taxol	16.9	42.7

*Each 45-μl reaction mixture contained 10 μg of tubulin, 5 μmols of glutamate, 50 nmols of $MgCl_2$, and indicated concentration of taxol (taxol concentration applies, however, to fin reaction volume of 50 μl). If indicated, reaction mixtures wer preincubated for 1 hr at 37°C and then chilled on ice. A 5-μ sample containing 0.1 μM [α-^{32}P]GTP was added to each reactio mixture. Samples were left on ice for 18 hrs and then analyze for GTP hydrolysis.

RESULTS AND DISCUSSION

Our initial efforts were directed at maximizing the sensitivity of the assay for taxol and minimizing the baseline reaction. The best results were obtained using 1.0 M glutamate, 1 mM $MgCl_2$, and 0.2 mg/ml of tubulin and a 0°C incubation.

We have found that 1.0 M glutamate induces a GTP-dependent, temperature-dependent polymerization of purified tubulin coupled initially to stoichiometric GTP hydrolysis (6). Previously we had observed no GTP hydrolysis at 0°C, but easily detectable GTP hydrolysis occurred at 37°C with high specific activity [α-^{32}P]GTP at nucleotide concentrations unable to support tubulin polymerization (6). With the addition of taxol, tubulin polymerization will occur at 37°C without GTP or at 0°C provided GTP is present (5). This taxol-dependent polymerization in 1.0 M glutamate at 0°C is associated with GTP hydrolysis [unpublished observations; (5)]. Measurement of tubulin-dependent GTP hydrolysis at 0°C in 1.0 M glutamate therefore provided a useful assay system for taxol, since there is a minimal reaction in the absence of the drug. Finally, we found that tubulin-dependent GTP hydrolysis in 1.0 M glutamate is not affected by dimethylsulfoxide concentrations as high as 30% (7). This is important since taxol is poorly soluble in water but readily soluble in dimethylsulfoxide.

Taxol stimulation of tubulin-dependent GTP hydrolysis at 0°C is significantly enhanced by preincubating taxol and tubulin at 37°C in the absence of GTP (table 1). Since this probably results in the formation of stable polymerization products prior to the addition of GTP (2,4,5), it is most likely these tubulin polymers that are responsible for most of the hydrolytic reaction. Because of the significant enhancement of the taxol-stimulated reaction, with no effect on the baseline, this drug-tubulin preincubation at 37°C was performed in all experiments presented here. With the prolonged 0°C incubation of this experiment, there was definite, although slight, hydrolysis of GTP by tubulin alone.

Sensitivity for taxol could also be enhanced if high specific activity [α-^{32}P]GTP was used (fig 1). The difference between 0.01 and 0.1 μM GTP is not substantial and probably not significant, but at these nucleotide concentrations sensitivity for taxol is substantially greater than at 10 μM GTP. Sensitivity is also enhanced at the lower GTP concentrations by the greater range of the reaction, the plateau occurring when > 90% of the GTP is hydrolyzed rather than 40% as occurs with 10 μM nucleotide.

Figure 2 demonstrates the reproducibility of the assay, using 0.01 μM GTP. Three separate experiments were performed, with all experimental points representing an average of nine values. The inset demonstrates that sensitivity was at least as low as 0.1 μM taxol in two of these experiments and 0.2 μM taxol in the third. Figure 3A shows an autoradiogram demonstrating the hydrolysis of GTP observed with 1 μM taxol.

We next examined the suitability of this assay for the determination of taxol levels in serum. Since the phosphatase activity of serum was substantial (fig 3A), methods of treating serum were evaluated for effectiveness in eliminating these enzymes while preserving the taxol content of serum. In these experiments exogenous taxol was added to human serum at 10 μM, and 10% treated serum was used in assays for a final nominal taxol concentration of 1 μM.

Heat and perchlorate treatment of serum were both effective in eliminating its phosphatase activity, but exogenously added taxol was also lost (data not presented). Similar results were obtained with ultrafiltration of serum (fig 3B, table 2), suggesting that taxol is largely bound by serum proteins.

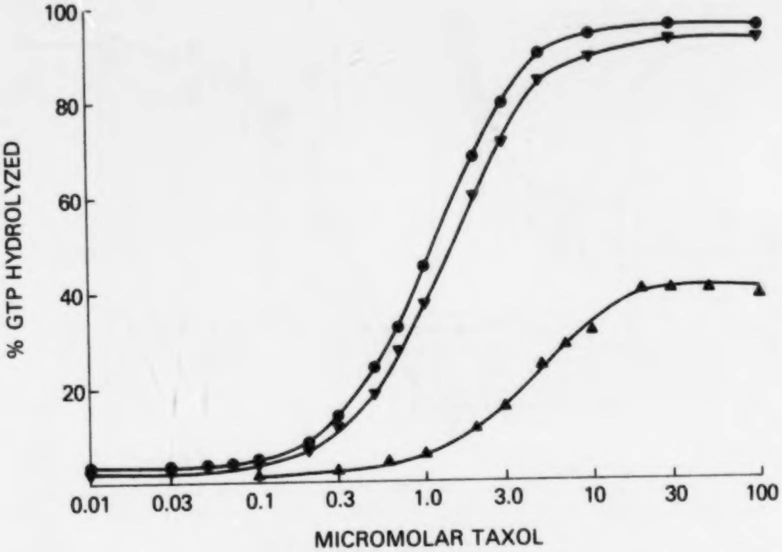

FIGURE 1.—Effect of increasing specific activity of [α-^{32}P]GTP on taxol-dependent stimulation of GTP hydrolysis by tubulin. Each 45-μl reaction mixture contained 10 μg of tubulin, 50 μmols of glutamate, 50 nmols of $MgCl_2$, and indicated concentration of taxol (taxol concentrations apply, however, to final reaction volume of 50 μl). Reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing [α-^{32}P]GTP of varying specific activity was added to each reaction mixture for final nucleotide concentrations of 10 μM (▲), 0.1 μM (▼), or 0.01 μM (●). Samples were left on ice for 18 hrs and then analyzed for GTP hydrolysis. Each experimental point was performed in triplicate, and each of these was analyzed in triplicate. Average of 9 values obtained is presented. Ranges obtained are similar to those presented in fig 2. Average % GTP hydrolyzed in absence of taxol was as follows: at 10 μM GTP, 0.9%; at 0.1 μM GTP, 2.4%; and at 0.01 μM GTP, 3.6%.

The most effective treatment we have thus far found consists of precipitation of serum proteins with four parts dimethylsulfoxide and their removal by centrifugation, followed by lyophilization of the supernatant and resuspension of the residuum in a volume of dimethylsulfoxide equivalent to the original serum volume (fig 3C, table 2). This essentially eliminates serum phosphatase activity and leads to the recovery of > 75% of the added taxol (average of four experiments was 80% recovery), presumably because of the high solubility of the drug in dimethylsulfoxide.

To demonstrate the usefulness of this assay for the determination of taxol levels in clinical specimens, the serum extraction method was used in a preliminary experiment to follow the pharmacokinetics of taxol in the rabbit, except that the lyophilized residua were dissolved in one half the original serum volumes to enhance sensitivity (fig 4). In this experiment, we attempted to minimize the effect of incomplete extraction of taxol: the standard curve (fig 4, inset) was generated from samples of baseline rabbit serum containing known concentrations of taxol that were extracted in the same way as the serum samples obtained after injection of the drug.

Figure 4 demonstrates that taxol, after rapid iv injection in a rabbit at a dose of 8.5 mg/kg, is rapidly cleared from the serum.[4] Assuming a two-compartment open model (8), its apparent α-phase half-life was 2.7 mins, and its β-phase half-life was 42 mins. At 2 hrs the serum level was < 3% of that found at the initial 1-min time point. An ultrafiltrate was also prepared from the serum sample obtained 5 mins after drug injection. This contained about 8% of the taxol found in the serum extract, comparable to the result shown in table 2 with exogenously added taxol (data not presented).

In summary, we have developed a biochemical assay for taxol with sensitivity to 0.1 μM. This assay exploits the ability of taxol to induce tubulin to form cold-resistant polymers that hydrolyze GTP at 0°C. We have found that taxol is almost totally protein-bound but is nevertheless cleared rapidly from se-

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[4] For comparison, an optimal therapeutic dose with mouse B16 melanocarcinoma was 5 mg/kg ip daily for 9 days (M. C. Lowe, personal communication).

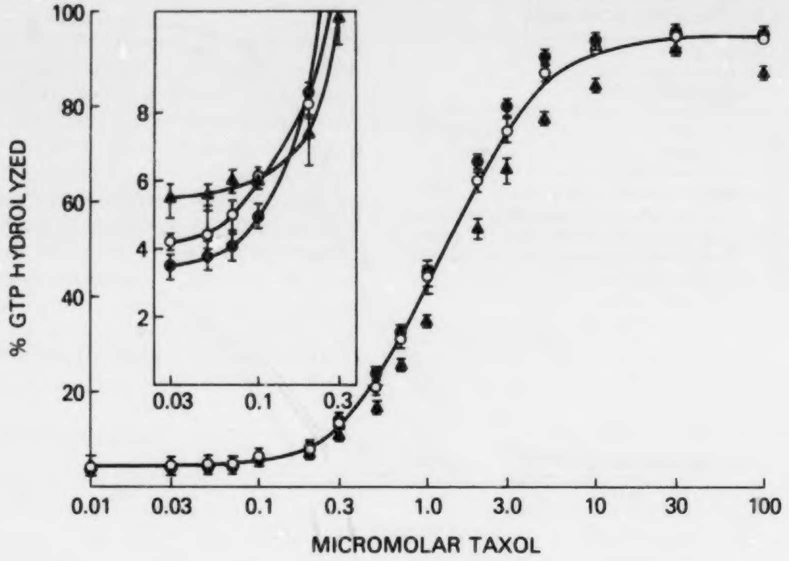

FIGURE 2.—Experimental reproducibility of taxol-dependent stimulation of GTP hydrolysis by tubulin at 0.01 μM GTP. Experimental conditions were identical to those described in fig 1, except that all 3 experiments were performed with 0.01 μM [α-^{32}P]GTP. Average % GTP hydrolyzed in absence of taxol was as follows: 1st experiment (●), 3.6% (range, 3.2%–3.9%); 2nd experiment (▲), 5.1% (range, 4.8%–5.3%); and 3rd experiment (○), 3.9% (range, 3.5%–4.2%). Inset presents data from 0.03–0.3 μM taxol on an expanded scale.

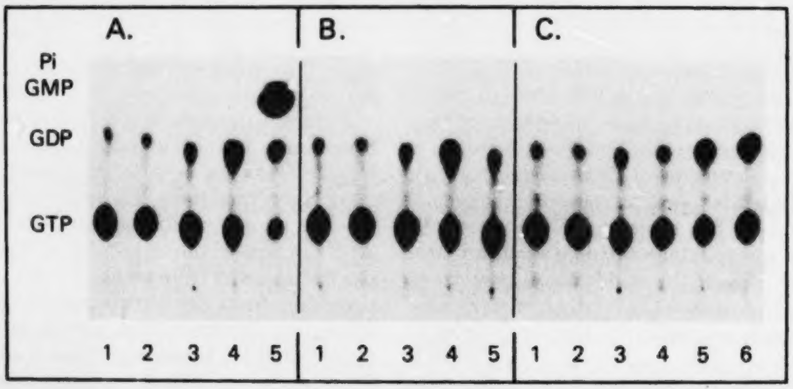

FIGURE 3.—Autoradiograms of reaction mixtures used to study effect of taxol on the GTPase activity of tubulin. Reaction conditions were identical to those described in table 2, with all mixtures containing GTP, glutamate, $MgCl_2$, and dimethylsulfoxide. A) Effects of tubulin, taxol, and serum. Additions to reaction mixtures were as follows: 1 = no addition; 2 = 1 μM taxol; 3 = 10 μg of tubulin; 4 = 10 μg of tubulin and 1 μM taxol; and 5 = 5 μl of human serum. B) Effect of serum ultrafiltration. Additions to reaction mixtures were as follows: 1 = no addition; 2 = 5 μl of serum ultrafiltrate; 3 = 5 μl of serum ultrafiltrate and 10 μg of tubulin; 4 = 10 μg of tubulin and 1 μM taxol; and 5 = 10 μg of tubulin and 5 μl of an ultrafiltrate of serum containing 10 μM taxol. C) Effect of dimethylsulfoxide extraction of serum. Additions to reaction mixtures were as follows: 1 = no addition; 2 = 5 μl of serum extract; 3 = 10 μg of tubulin; 4 = 10 μg of tubulin and 5 μl of serum extract; 5 = 10 μg of tubulin and 1 μM taxol; and 6 = 10 μg of tubulin and 5 μl of an extract of serum containing 10 μM taxol, with extract having same volume as original serum.

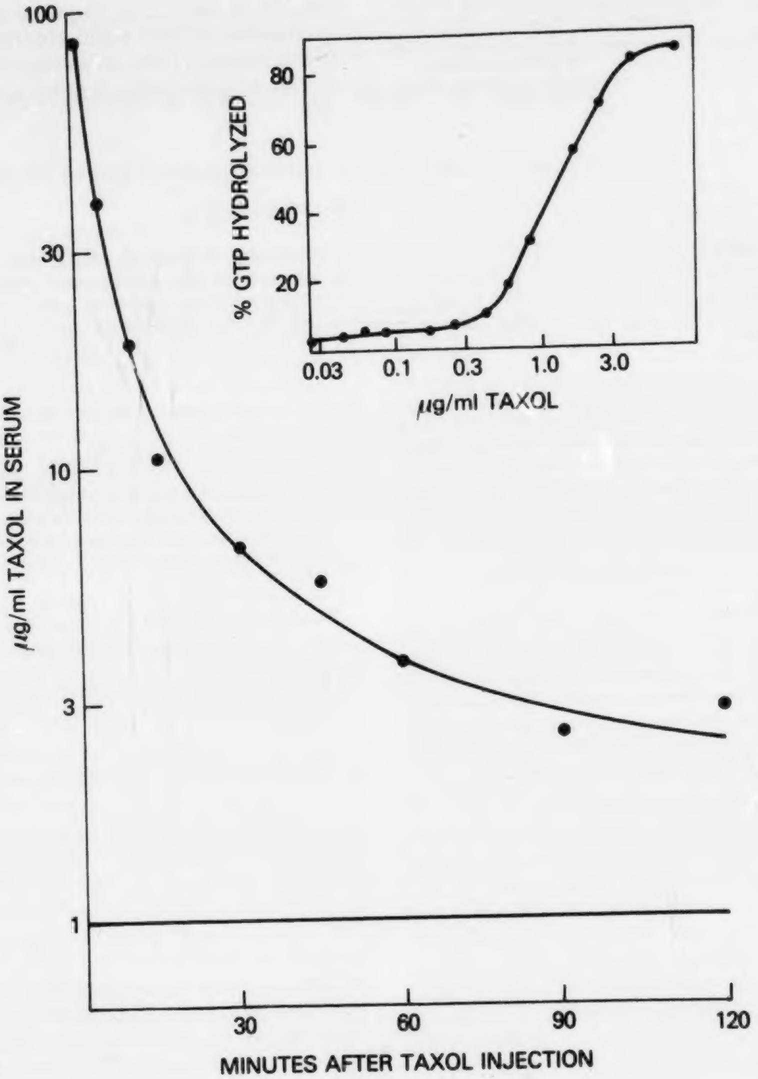

FIGURE 4.—Taxol pharmacokinetics following bolus iv injection. A 3.5-kg rabbit was anesthetized by iv injection of 180 mg of sodium pentobarbital, and femoral artery and vein were cannulated. A total of 1.75 ml of dimethylsulfoxide containing 17 mg of taxol/ml (0.02 M) was injected iv over 30 secs, and 1-ml blood samples were drawn at indicated times. Serum was prepared from blood samples, mixed with 4 parts dimethylsulfoxide, and centrifuged for 5 mins at 20,000 × *g*. Protein pellets were discarded, and 0.75 ml of each supernatant was lyophilized. Residua were dissolved in 0.075 ml of dimethylsulfoxide, and resulting solutions were used to determine the taxol content of serum. Each 45-μl reaction mixture contained 10 μg of tubulin, 50 μmols of glutamate, 50 nmols of $MgCl_2$, and 5 μl of serum extract or 5 μl of a mixture of an extract of baseline serum and extract of serum obtained at indicated time. Reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing 0.1 μM [α-^{32}P]GTP was added to each reaction mixture. Samples were left on ice for 18 hrs and analyzed for GTP hydrolysis. Data in this figure are presented in μg/ml rather than as molar concentrations because drug appears to be largely protein-bound (0.85 μg/ml is equivalent to 1 μM). Minimum detectable level of taxol was about 1 μg/ml. Inset: standard curve used to generate data of main portion of figure. Known amounts of taxol were added to aliquots of rabbit serum that were extracted and assayed as described above. Values on abscissa refer to original concentrations in serum.

TABLE 2.—Recovery of exogenously added taxol from human serum*

	% GTP hydrolyzed
+ Tubulin only	3.1
+ Tubulin + 0.7 μM taxol	22.3
+ Tubulin + 1.0 μM taxol	34.1
+ Serum ultrafiltrate	0.3
+ Tubulin + serum ultrafiltrate	3.4
+ Tubulin + ultrafiltrate of serum containing taxol (final concentration nominally 1.0 μM)	3.8
+ Serum extract	0.8
+ Tubulin + serum extract	5.4
+ Tubulin + extract of serum containing taxol (final concentration nominally 1.0 μM)	25.3

*Each 45 μl of reaction mixture contained 50 μmols of glutamate, 50 nmols of $MgCl_2$, and the following, as indicated: 10 μg of tubulin, taxol as indicated (concentrations applying to final reaction volume of 50 μl), 5 μl of serum ultrafiltrate, 5 μl of ultrafiltrate of serum containing 10 μM taxol, 5 μl of extracted serum, and 5 μl of extract of serum containing 10 μM taxol. Reaction mixtures were preincubated for 1 hr at 37°C and then chilled on ice. A 5-μl sample containing 0.1 μM [α-^{32}P]GTP was added to each reaction mixture. Samples were left on ice for 18 hrs and analyzed for GTP hydrolysis. Serum ultrafiltrates were prepared with Amicon MPS-1 micropartition filtration devices. Extracted serum was prepared by mixing 0.5 ml of serum with 2.0 ml of dimethylsulfoxide. Precipitate was removed by centrifugation at 20,000 × *g*, and 1.6 ml of the supernatant was lyophilized. Residuum was dissolved in 0.32 ml of dimethylsulfoxide. Serum containing taxol was prepared by mixing 0.495 ml of serum with 5 μl of 1 mM taxol. Ultrafiltration and extraction were performed as described above.

rum. It is obviously important to determine the mechanism of this rapid clearance for the appropriate design of chemotherapeutic regimens. Such studies are currently being planned in our laboratory.

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Workshop on Kaposi's Sarcoma: Meeting Report

William D. DeWys,[1,*] James Curran,[2] Werner Henle,[3] and Gerhard Johnson[4]

On July 3, 1981, investigators from the Centers for Disease Control (CDC) reported a clustering of cases of Kaposi's sarcoma (KS) in homosexual men in New York City and California (1). KS is a rare tumor in North America and Europe and usually occurs in aged men (mean age, 63 years). The 26 cases reported occurred in younger men (range, 26–51 years; mean, 39), and seven cases also had serious infections including *Pneumocystis carinii* pneumonia (PCP), CNS toxoplasmosis, extensive candidiasis, and cryptococcal meningitis. All patients tested had serologic evidence of past or present cytomegalovirus (CMV) infection.

Based on this report and a subsequent followup (2), the Division of Cancer Treatment and the Division of Cancer Cause and Prevention of the National Cancer Institute (NCI) and the Task Force on Kaposi's Sarcoma and Opportunistic Infections of the CDC sponsored a Workshop on Kaposi's Sarcoma on September 15, 1981, at the National Institutes of Health.

At the workshop, **J. Curran**, CDC, reviewed the series of cases reported to the CDC to date (1–3), which included 55 cases of KS, 49 cases of PCP, and 11 cases of both diseases, either concurrently or sequentially. All cases were in men, 94% of whom were homosexual or bisexual. Eighty percent of these patients were between 25 and 45 years of age. The classic dark blue or violaceous plaques or nodules on the skin or mucous membranes were present in most cases, but other frequent presenting signs and symptoms included lymphadenopathy, fever, diarrhea, and weight loss. More than 85% of the men resided in New York City, Los Angeles, or San Francisco, which correlated with known concentrations of homosexual men. Since one would expect ≤ 100 cases of KS in the US per year, these observed cases in homosexual men suggest an epidemic of this disease in this population. Although preliminary epidemiologic observations suggest a correlation of KS with sexually transmitted diseases, increased numbers of sexual partners, and use of nitrite inhalants, investigators from CDC have initiated a detailed case-control epidemiologic study to more clearly identify and delineate these risk factors.

Ziegler, Vogel, and **Templeton** reviewed the natural history of nonepidemic KS. Four clinical categories have been described. The *nodular* form generally is chronic, indolent, and associated with some degree of edema, appearing in most cases on the extremities; persons with nodular KS may not seek medical attention unless the nodules ulcerate, become somehow incapacitating, or begin to invade deeper tissues. This is the predominant form in elderly men. The *florid* form features an exophytic sarcomatous transformation of a nodule which then becomes locally aggressive and invasive. In the yet more serious *infiltrative* form of KS, the tumor penetrates deeply and very often will involve underlying bone. The florid and infiltrative forms are seen predominantly and endemically in equatorial Africa. Finally, the *generalized* form has been termed lymphadenopathic because it often presents with multiple lymph node enlargements; the disease is primarily associated with mucosal lesions, often becoming disseminated through the lungs and gastrointestinal tract, and the prognosis is poor because of the generalized aggressive nature of this form of KS. This is the form commonly seen in the current US epidemic and in African children and young adults.

The nonendemic form of KS occurs predominantly in older males, with an apparent predilection for persons of Jewish or Italian ancestry. The endemic incidence is 100-fold greater than the nonendemic incidence. An increased incidence of KS occurs in recipients of renal allografts, a median of 16 months after allografting. If immunosuppression is withdrawn, some Kaposi lesions regress.

KS may have several patterns of dissemination. The most common pattern is regional development of multiple cutaneous and subcutaneous nodules. Spread to regional nodes may occur, but this must be differentiated from inflammatory lymphadenopathy secondary to tumor ulceration and infection. Involvement of multiple mucosal surfaces through-

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[1] National Cancer Institute, Bethesda, MD.
[2] Centers for Disease Control, Atlanta, GA.
[3] Children's Hospital of Philadelphia, PA.
[4] Veterans Administration Medical Center, Minneapolis, MN.
[*] *Reprint requests to:* Dr. William D. DeWys, Clinical Investigations Branch, Landow Bldg, Rm 4A04, National Cancer Institute, Bethesda, MD 20205.

TABLE 1.—Results of treatment in Ugandan patients with KS

Drug regimen	No. of evaluable patients	Response		
		Complete	Partial	None
Dactinomycin	12	2	7	3
Cyclophosphamide	10	1	0	9
Dactinomycin + vincristine	14	10	3	1
Dactinomycin + vincristine + dacarbazine	32	30	1	1
Data compiled from the literature for other agents				
BCNU	21	4	5	12
Bleomycin	10	0	6	4
Dacarbazine	10	0	5	5
MOPP	5	0	0	5
Daunorubicin	3	0	0	3
ICRF-159	47	10	17	20

out the airway and gastrointestinal tract is a unique feature of this disease. Finally, visceral metastases, especially to lung, may develop. Based on these patterns, evaluation of extent of disease in KS may require lymph node biopsy, laryngoscopy, and gastrointestinal endoscopy in addition to the usual clinical examinations, radiographs, and scans.

G. Giraldo described studies on the possible role of CMV in KS. Antibody titers to CMV were higher in KS patients compared to controls in studies in the US and Italy, but titers in African patients did not differ significantly from African controls (African controls also had elevated titers). In recent work, Giraldo (4) has been able to identify CMV DNA and gene products in about one third of KS tissues using several techniques. **L. Drew** reported high titers of IgG antibodies to CMV in homosexual men. **Friedman-Kien** reported decreased helper T lymphocytes and increased T suppressor cells in homosexual patients with KS, as studied with monoclonal antibodies. However, studies of T-cell function were not reported. HLA typing of KS patients by **P. Rubenstein** showed 61% having the DR-5 antigen, compared with 19% in a control population.

Ziegler reviewed the results of treatment in Ugandan patients as shown in table 1.

Laubenstein reported on a trial of doxorubicin, bleomycin, and vinblastine, which has produced partial remissions in all nine patients treated so far. KS is also radiosensitive, and wide-field radiation therapy merits further evaluation.

After the above presentations and discussions, the workshop participants divided into three groups to develop recommendations for future studies.

Epidemiology

The group considering epidemiology, chaired by J. Curran, reported that a national case/control study of KS and/or PCP among homosexual men will be conducted by CDC, and they stated that it would be desirable to have laboratory studies coordinated with this study. They recommended national retrospective and prospective active surveillance of KS and PCP in the US. The following suggestions for future studies were also made:

(*a*) Definition of the magnitude and nature of the "immunosuppressive syndrome" among homosexual men. How large is the group at risk for KS and/or PCP?

(*b*) Examination of recent past and future cancer incidence data to detect an excess of other cancers among homosexual men (or single men) especially from geographic areas such as New York City and San Francisco. Special attention should be directed to tumors associated with immunosuppression.

(*c*) Use of a *retrospective cohort* approach to study illness incidence among homosexual men. The participants in the CDC and New York Blood Center hepatitis B vaccine trials have been characterized by antigen and behavioral studies and could be contacted for followup.

(*d*) An epidemiologic study of elderly men with Kaposi's sarcoma, examining sex preference, marital status, exposure to various nitrites, etc.

(*e*) Prospective followup of immunosuppressed homosexual men at risk for developing KS or PCP.

Virology-Etiology

The session on virology-etiology, chaired by **W. Henle** concluded that at present, CMV is the leading candidate for an etiologic role in KS; recommendations for future studies are heavily oriented toward this virus. While evidence for a relationship between CMV and KS has been steadily mounting, due largely to the efforts of Giraldo and his associates, there are some as yet unresolved discrepancies, such as the difference in antibody responses to CMV between white KS patients (elevated titers) and African patients (not different from controls) and the absence of CMV "fingerprints" in some of the KS biopsies that have been tested. It is essential, therefore, to keep an open mind and to look for other viruses that might be associated with the tumor. The studies recommended would detect not only CMV but other viruses or viral products.

Of primary importance is the collection of appropriate, well-characterized specimens from KS patients and controls in a coordinated manner. A central depository from which qualified investigators could draw needed specimens would be most desirable. Thus, aliquots of specimens would be tested in several laboratories by possibly different techniques permitting comparisons and correlation of results. The specimens to be collected include biopsy material (frozen or fresh) and sera.

Biopsy material.—Frozen biopsy specimens are needed for the detection of viral nucleic acids by appropriate hybridization techniques, including in situ tests to identify the specific type of cells harboring viral genomes. Fresh biopsy specimens are needed for initiation of cultures, making of touch preparations (which after fixation can be stored frozen), and transplantation to nude (athymic) mice to separate the malignant (growing) from normal (not growing) cells. The materials should be examined for the presence (or emergence) of viruses, viral antigens, or viral nucleic acids by pertinent techniques: eg, virus isolation and identification; and immunofluorescence (IF) by the indirect and anti-C′ techniques using virus-specific reference sera and virus-specific nucleic acid hybridization tests. It will be essential to obtain and test control specimens also; ie, biopsy material from apparently normal tissue adjacent to KS lesions. When found histologically free of KS, these specimens should be tested in parallel with the KS specimens of the same patient to differentiate between strictly tumor-associated virus or viral products and persistent latent, perhaps activated, infections in anatomic regions serving as a natural habitat for the virus.

Sera.—These should be collected from:

(*a*) KS patients before initiation of therapy and serially thereafter at intervals of 3–4 months or more frequently based on clinical events.

(*b*) Homosexual men without KS but having a lifestyle similar to that of KS patients. If CMV antibody-negative individuals are identified, they should be followed prospectively, if possible, to determine their immune responses to primary CMV infections when they occur.

(*c*) Healthy controls matched for age, sex, race, and socioeconomic status.

(*d*) Patients with primary CMV infections and patients with recurrent CMV infections.

Several different serologic tests are being used for measuring antibodies to CMV. Of these, IF has the advantage of visually differentiating between various distinct virus-coded antigens by their cellular localization and the morphology of staining. IF also can identify the immunoglobulin class of the reactive antibodies. Most other techniques require separation and a high degree of purification of CMV-coded antigens, which are not readily accomplished.

The preparation of cell smears for measuring antibody titers to various CMV-coded antigens by indirect or anti-C′ IF is presently cumbersome and not amenable to mass production, since it depends on cells grown and infected on coverslips. Efforts should be made to find cells that grow in suspension cultures and are capable of synthesizing the various CMV-coded antigens under specified conditions. Cell smears could then be made which should be uniform with respect to the number and distribution of antigen-containing cells.

The virus-specific antibody spectrum and titers should be determined for patients with primary or recurrent CMV infections, healthy or immunologically compromised carriers of the virus, and, of course, KS patients at various stages of the disease. There could conceivably be an antibody pattern characteristic of KS which could serve as a diagnostic aid. Also, long-term serologic followup studies on KS patients might reveal changes in the spectrum and titers of antibodies which might reflect the prognosis (decreases being favorable and increases unfavorable) as observed with the Epstein-Barr virus-specific antibody patterns in Burkitt's lymphoma and nasopharyngeal carcinoma.

If and when other candidate viruses for an etiologic relation to KS are found, they must be subjected to the same type of serodiagnostic and seroepidemiologic studies.

While the above approaches might implicate CMV or another virus in the etiology of KS, this alone would not suffice to explain completely the present epidemic of the disease in homosexual men. The key questions remain as to what causes the extreme immunodeficiency in the patients and what comes first, KS or the immunosuppression. Probably the immune deficiency precedes KS. The various drugs, such as amylnitrite and butylnitrite, which have been used excessively by many homosexual KS patients, should be tested in animals for their immunosuppressive, mutagenic, and oncogenic potential.

Aggressive chemotherapy and persistent infections, such as CMV, Epstein-Barr virus, hepatitis B, pneumocystis, and amebiasis, likely contribute to the final degree of immunologic incompetence. Careful evaluation of the immune reactivities of KS patients before therapy and continuing thereafter is considered essential. It was pointed out that in addition to identifying various types of leukocytes by monoclonal antibodies, their functional state must also be ascertained.

Treatment

The working group considering treatment of KS agreed on a general format for any prospective study. Components of the protocol would include eligibility, pathology confirmation, staging, immunologic evaluation, infection prophylaxis, and treatment.

Eligibility.—Although trials would be primarily designed to offer a systematic approach to the treatment of the aggressive form of KS in male homosexuals, all patients with a diagnosis of KS would be eligible for entry into such a study.

Pathology confirmation.—A Central Pathology Review Panel should be established to review the tissues obtained from all patients entering clinical trials.

Staging.—Patients may be staged according to the scheme:

Disease location	Disease activity*	
Local/regional	Indolent	Aggressive
Generalized	Indolent	Aggressive

*Designation of aggressive disease is to be based on tempo of disease and involvement beyond skin and mucosal surfaces.

The following staging evaluation was recommended:

(*a*) Laboratory studies: hemoglobin, leukocyte count, differential, platelet count, reticulocyte count, erythrocyte sedimentation rate, serum protein electrophoresis, quantitative immunoglobulins, immunoelectrophoresis, VRL, serum creatinine, uric acid, bilirubin, SGOT, alkaline phosphatase, electrolytes, and calcium and phosphorus.

(*b*) X-ray studies: chest X-ray, upper gastrointestinal series with small bowel follow-through, barium enema, computerized axial tomographic scan of abdomen and pelvis.

(*c*) Radioisotope scans: liver/spleen scan, bone scan if alkaline phosphatase of bone origin is elevated.

(*d*) Endoscopy: upper gastrointestinal endoscopy and colonoscopy (bronchoscopy should be performed if the chest X-ray is abnormal and there is reason to suspect infection).

Immunologic evaluation.—Delayed hypersensitivity should be evaluated with a battery of skin tests, including *Candida*, *Trichophyton*, mumps, and purified protein derivative. Additional immunologic studies are to be defined.

Infection prophylaxis.—In view of the immunosuppression and propensity to infection with *Pneumocystis carinii* and other opportunistic infections, all patients should be treated prophylactically with trimethoprim and sulfamethoxazole.

Treatment.—Treatment assignment should be based on the patient's clinical stage of disease as defined above. Possible regimens are based on cumulative experience with this disease and pilot studies currently in progress at New York University, the University of California at San Francisco, and Memorial Sloan-Kettering Cancer Center.

For both indolent and aggressive local/regional disease, patients may be treated with regional radiation therapy. For indolent generalized disease, patients may be treated with vinblastine, ICRF-187[5] or VP-16-213.[6] For aggressive generalized disease, combination chemotherapy[7] may be used.

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[5] Patients without liver abnormalities (normal bilirubin and negative computerized tomographic scan of the liver) can receive 2.5 g/m²/wk, iv; patients with liver involvement, 1.5 g/m²/wk, iv. Treatment would be given weekly for 4 wks followed by a 2-wk rest period. If moderate myelotoxicity is not observed, a 20% dose escalation can be used. Appropriate dosage reduction should be made for patients who develop cytopenias.

[6] Patients may receive 150 mg/m²/day, iv, for 3 days; cycles can be repeated every 3–4 wks.

[7] Dactinomycin (1.5 mg/m², iv) on Day 1, dacarbazine (750 mg/m², iv) on Day 1, and vincristine (2 mg, iv) on Days 1 and 15 can be given, as can doxorubicin (40 mg/m², iv) on Day 1, vinblastine (6 mg/m², iv) on Day 1, and bleomycin (15 units, iv) on Days 1 and 15. Patients who fail one regimen might cross over to the other combination.

The Epidemic of Acquired Immune Dysfunction in Homosexual Men and Its Sequelae—Opportunistic Infections, Kaposi's Sarcoma, and Other Malignancies: An Update and Interpretation

Arthur S. Levine[1,*]

Since the original report of Gottlieb et al (1) on five cases of *Pneumocystis carinii* pneumonia (PCP) in young homosexual men from California, the report 1 month later of 26 cases of Kaposi's sarcoma (KS) occurring in a similar population (2), and the workshop held at the National Institutes of Health in September of 1981 (*see* companion report), much new information has become available. The following summary of that new information is abstracted from a second workshop (held at the Centers for Disease Control [CDC], March 3, 1982), from recent reports in the literature or in press, and from personal communications.

INCIDENCE AND SURVIVAL

At the time of this writing, 300 cases of the new syndrome have been reported, 3% of which were diagnosed in 1979, 11% in 1980, and 86% since January 1981. Of these 300 patients, 135 have had PCP; 115, KS; 28, both diseases; and 22, other opportunistic infections (3,4). The mortality rate so far has been 40%; however, > 70% of patients with the earliest diagnosed cases have died, predicting that the ultimate mortality will be staggering. Eighty-five percent of the 300 patients have been < 45 years old, with a median of 35.

Of these 300 patients, 290 have been men. Of these 290 men, 242 have been homosexual or bisexual, 30 have been heterosexual, and 18 have been men with an unknown sexual preference. Ten of the 300 patients have been women (predominately heterosexual). Of the 300 patients, 52% have been from New York City, 22% from California, 23% from 19 other states, and 3% from six foreign countries (a number of these foreign patients had recently been to the United States) (3).[2] Of interest are a cluster of 11 cases reported from Haiti and a cluster within a prison population. On the average, one new case of this syndrome is being reported each day. Considering all of the epidemiologic observations together, a growing accumulation of risk factors is suggested rather than the spread of disease in the classic sense of contagion.

Forty-five percent of the 242 homosexual male patients have developed KS; 97% of all KS cases in this epidemic have occurred in homosexual males. Seventy-three percent of the PCP cases have occurred in homosexual males, and 38% of the homosexual male patients have developed this infection. Only 12% of the heterosexual male and the female patients have developed KS, but 72% of this group have developed PCP. Seventy-five percent of the homosexual male patients have been white, with the rest evenly divided between black and Hispanic; only 20% of the heterosexual male and the female patients have been white. As with the homosexual male patients, the heterosexual male and female patients have been concentrated in New York and California (3).

RISK FACTORS

The CDC Task Force has compared homosexual male patients with clinically normal homosexual and heterosexual male controls. These controls have been matched to the patients for age, race, and city of residence. Among homosexual males, a history of gonorrhea, hepatitis B, amebiasis, and/or cytomegalovirus (CMV) infection is very much more common than among heterosexual men; all of these infections, especially CMV, are virtually endemic in the homosexual male population. However, when patients with this syndrome are matched to homosexual controls, the only factors that are somewhat more prominent in the patients are the frequency of CMV isolation from the urine and a CMV antibody titer > 1:128; an elevated Epstein-Barr virus (EBV) titer; a positive serologic test for syphilis; and enterovirus isolation. KS cases tend to have elevated plasma IgG levels, and PCP cases elevated

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[1] Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[2] Minutes of a workshop held at the CDC, Atlanta, GA, March 3, 1982 (unpublished).

Reprint requests to: Arthur S. Levine, MD, Division of Cancer Treatment, National Cancer Institute, Bldg 31, Rm 3A49, Bethesda, MD 20205.

IgA levels, as compared with matched homosexual controls. The median number of lifetime male sexual partners for homosexual male patients is 1160 versus 524 for matched homosexual controls. Moreover, the number of "bathhouse partners" within the past year is increased in patients versus matched controls.[2]

Ninety-eight percent of the homosexual male patients have had a history of exposure to nitrites, mainly amyl or butyl nitrite inhalants, but no one nitrite preparation is over-reported, suggesting that whatever the involvement of nitrites in this syndrome, that involvement is not due to a contaminant.[2] A history of nitrite usage is just as prevalent in the matched male homosexual control population, with > 85% of apparently healthy male homosexuals in New York and California reporting such use during the past 5 years. Of the heterosexual male and the female patients, only 14% have had a history of nitrite exposure, not significantly different than the incidence in a matched heterosexual control population; however, 60% of the heterosexual male and female patients have had a history of intravenous drug abuse, mainly involving opiates (3). Nitrite use correlates closely with the number of sexual partners and even with specific homosexual practices, specific social events, and specific meeting places. Therefore, a very extensive case-control study will be required to sort out the relative contributions of nitrites and transmissible microbes; in fact, it may be impossible to identify independent risk factors. However, it is clear that the syndrome is occurring mainly in a particular subset of the homosexual male population, possibly but not exclusively defined by the number of sexual partners.

It is of great interest that nitrites are the only drug the use of which has sharply increased in the US during the 1970s.[2] Of interest also is the fact that the nonmedicinal-grade nitrites now in use were first manufactured in California and then shipped to New York City; at present they are virtually ubiquitous.

IMMUNE DYSFUNCTION

There is evidence that a very sizable number of apparently healthy homosexual men in the US, especially those residing in New York and California, now have an acquired immune dysfunction. Goedert et al (4) have reported that seven of eight unselected and clinically normal homosexual men with a history of nitrite usage had a low T lymphocyte helper/suppressor (T-H/S) ratio, with OKT8+ cells increased in number, as detected by monoclonal antibody, and OKT4+ cells reduced. In cases of the new syndrome, the T-H/S ratio has been about 0.7 versus 1.8 in controls (5–8).[2] The reduction in helper cell numbers is absolute, but the increase in suppressor cell numbers is usually relative (although some patients have had a modest absolute increase). In the study by Goedert et al, only one of seven unselected and clinically normal homosexual males without a history of nitrite usage had a low T-H/S ratio. Of all 15 clinically normal male homosexual volunteers studied by Goedert et al (4), 14 had high CMV titers, and there was no correlation between CMV titers and nitrite use or T-H/S ratios. Thus, CMV alone does not produce the inverted T-H/S ratio, but nitrites may. One can extrapolate from these findings to the prediction that tens of thousands of homosexual men may have the acquired immune dysfunction and be at risk for development of the clinical syndrome. Indeed, we very probably have seen only the tip of the iceberg.

In addition to the low T-H/S ratio, other findings in patients with this syndrome have included cutaneous anergy, lymphopenia (often profound), leukopenia, diminished in vitro lymphoproliferative responses to soluble antigens and phytohemagglutinin (two thirds of the cases have had this in vitro anergy), and diminished natural killer cell activity despite an increase in the number of OKT8+ cells (the latter has not been a consistent finding) (5,6). The number and function of B lymphocytes have been intact in patients, and often, as noted previously, the level of immunoglobulins has been increased. In fact, it is now apparent that some patients demonstrate autoimmune phenomena rather than KS or opportunistic infections (9).[2] These patients, with increased OKT4 positivity, have had manifestations of lupus, acquired autoimmune hemolytic anemia, or autoimmune thrombocytopenic purpura. It may be that this patient subset is evidencing polyclonal B cell activation early in the evolution of their syndrome.

The breadth of immune defects seen in this syndrome certainly explains the broad gap in host defenses against all classes of microbes that are associated with cellular immunity. The degree of immunodeficiency is extraordinary, and not approached even in patients treated chronically with cyclosporin A, cyclophosphamide, or steroids. Thus, this is the first documented epidemic of community-acquired immune dysfunction. The KS and opportunistic infections appear to be secondary to the acquired immune dysfunction, as occurs in renal transplant patients or patients with autoimmune disorders treated chronically with immunosuppressive agents. In these latter types of patients, when the immunosuppressive treatment is discontinued, the KS lesions may regress, again suggesting that the immunodeficiency is primary and the malignancy and infections are secondary.

It is also of interest in this regard that renal transplant patients and patients treated chronically with immunosuppressive agents tend to have the generalized and aggressive form of KS, as do patients in the current epidemic (10). Moreover, KS patients, whatever the setting, have an increased incidence of lymphoma. Finally, the latency for appearance of KS in renal transplant patients is about 1½–5 years, which would appear to be consistent with the emerging picture of the present epidemic.

CYTOMEGALOVIRUS

As noted previously, CMV is endemic in the homosexual male population, being sexually transmitted via the urine and semen (11). Again, there is no evidence that primary infection is more common in patients with this new syndrome than in matched homosexual controls, but it is likely that once the patients have become immunodeficient, there is considerable reactivation of the CMV infection. CMV is itself immunosuppressive and can reduce the T-H/S ratio for up to 10 months after acute CMV mononucleosis in humans, as well as in the mouse model (11). However, as discussed above, the homosexual volunteers in the study by Goedert et al, with high CMV titers but no history of nitrite usage, did not show an altered T-H/S ratio. Therefore, it is probable that following the primary induction of immune dysfunction, a secondary CMV reactivation may lead to an increasingly profound immune deficiency as well as chronic antigenic challenge.

CMV is also a transforming virus in vitro; CMV DNA and antigens have been detected in a minority of KS tumors, and the virus has been isolated from a KS cell line (12). However, in the current epidemic, the CMV strains so far isolated show a diversity of restriction endonuclease patterns, and no new single mutant has emerged. While CMV has tropism for endothelial cells (the apparent cell of origin in KS), nothing precludes the possibility that CMV is merely a passenger in KS cells and not a primary oncogenic agent.

GENETICS

As noted in the accompanying report by DeWys et al, there is an association between the HLA-DR5 locus and the occurrence of KS in sporadic cases. This association is also suggested in the current epidemic, but there is no such association in patients who have PCP without KS (3).[2] Moreover, there may be an increased proportion of KS patients with Italian ancestry in the current epidemic, as is also true in sporadic cases, but again, these genetic associations are tentative at this time.

COURSE OF THE ILLNESS

It is now clear that this new syndrome reflects a broad spectrum of events, beginning with an asymptomatic acquired immune abnormality. In a small subset of the affected population, this abnormality may eventuate in autoimmune phenomena, but in most cases, the abnormality is an asymptomatic immune deficiency. Further along the spectrum is a prodromal syndrome with fever of unknown origin, diarrhea, weight loss, malaise, thrush, and wasting (5–7). In some respects, this prodromal syndrome is similar to that induced by a chronic viral infection or by graft-versus-host disease. Lymphadenopathy may or may not occur as an aspect of this prodrome; when it does, biopsy reveals reactive hyperplasia. The prodrome may last for weeks to months, and patients with lymphadenopathy may be stable for long periods. The most threatening end of the spectrum involves opportunistic infections and/or KS, and once any serious opportunistic infection has occurred, the course of these patients has been inexorably progressive (5–7).[2] The percentage of patients in whom the more benign events in this spectrum will lead to the more serious events is uncertain.

During the present epidemic, PCP has responded sluggishly and incompletely in most patients to TMP-SMZ and/or pentamidine. Moreover, there is a tendency for these patients to become severely leukopenic during treatment with TMP-SMZ even when they are given folates (6). Although TMP-SMZ prophylaxis has been recommended if laboratory findings suggestive of this syndrome are made, there is a question of whether the prophylactic regimen will be tolerated. With respect to other opportunistic infections, they reflect all classes of microbes associated with defective cellular immunity, including mycobacterial infections; interestingly, patients with mycobacterial infections have shown little or no granuloma formation (6).

As many as one third of KS patients in the epidemic appear to be developing other malignancies, as occurs with other KS patients, including the sporadic and endemic cases and the cases in renal transplant recipients or in patients receiving chronic immunosuppressive therapy (13). In the current epidemic, a number of patients have developed undifferentiated B cell lymphomas (apparently Burkitt's lymphoma). In three such patients, the tumors have been associated with EBV and one of the three with CMV as well (J. Ziegler, personal communication). Other tumors now being seen include squamous cell carcinoma of the tongue and cloacogenic carcinomas. These tumor types have always occurred among male homosexuals, but the incidence appears to be significantly increased during the current epidemic (3).[2]

TREATMENT

To date, only about 15% of patients with generalized KS treated during this epidemic with chemotherapy (usually doxorubicin, bleomycin, and vinblastine) have had a complete response, as contrasted with the 90% complete response rate in equatorial Africa. Moreover, because of the tendency toward leukopenia in this new syndrome, these patients have not often been able to tolerate aggressive chemotherapy.[2]

In addition to chemotherapy and radiotherapy, or in lieu of them, KS cases have been treated in the current epidemic with thymosin, interferon, allogeneic bone marrow transplantation, and/or acyclovir (5–8).[2] Thymosin has yielded no tumor responses; there appear to be partial responses to interferon; acyclovir has produced no tumor responses; and the single marrow transplantation of which we are aware is too recent to evaluate. The CMV infection has also not responded to acyclovir, as might have been predicted from earlier studies of this virus. As noted above, the response of PCP to therapy has often been unsatisfactory.

INTERPRETATION

All of the facts now available regarding this epidemic suggest that the primary event is an acquired immune dysfunction, possibly triggered by nitrites since they are the only apparently new risk factor. CMV, other known transmissible agents, and homosexuality per se are not new (although the very high median number of partners is also of recent vintage). Moreover, in preliminary experiments at the CDC, nitrites appear to induce immunodeficiency in mice. In one earlier small study, nitrites were believed to induce lymphomas, and in the Ames test, nitrites are mutagenic. With respect to heterosexual male and female patients not using nitrites but reporting the intravenous abuse of other drugs, it should be noted that marijuana and opiates have also been reported to be immunosuppressive, with an opiate affinity for T lymphocyte receptors (14). As with all diseases, there clearly must be a genetic predisposition to various aspects of this syndrome, with most people having no adverse effects from the accumulated risk factors, some demonstrating autoimmune phenomena, and a sizable minority manifesting the immunodeficiency. Genetic factors probably also condition whether once the immunodeficiency has occurred, people at risk will develop KS, other malignancies, opportunistic infections, merely the prodrome, or no illness at all.

It is almost certain that CMV is not the primary immunosuppressive agent but that reactivation of CMV in the setting of immunodeficiency produces still further immunosuppression. A great diversity of other sexually transmitted viruses, protozoa, bacteria, and parasites are also known to suppress transiently one or another element of the immune system,[2] and once infections with these agents occur, they too may contribute to an increasingly profound immunosuppression. It is also notable that semen itself is immunosuppressive, and among homosexual men, the immunosuppressive factor(s) in the semen might readily gain access to the circulation through the friable rectal mucosa (15). "Donor" lymphocytes in the semen might gain access in the same way and establish a graft-versus-host disease, possibly reflected in the wasting prodrome. Any role that the semen plays, however, is probably secondary since, again, these homosexual practices are not new. It has also been observed that when antigens are presented orally to the gut-associated lymphoid tissue, suppressor cells may disseminate widely from the gut- to non-gut-associated lymphoid tissue, inducing a specific hyporesponsiveness to that antigen (16,17). This phenomenon may also have a role in the complex syndrome. Finally, as the illness evolves, malnutrition and cachexia undoubtedly produce further immune deficiency.

In the setting of repeated antigenic challenge and severe, persistent immunosuppression, KS and other malignancies may develop just as in patients who have received kidney transplants or who for other reasons are treated chronically with immunosuppressive agents. It seems likely that CMV is a passenger within KS cells rather than a primary oncogenic agent. It is also possible that a retrovirus is involved in these malignancies; moreover, as a consequence of intercourse with animals, an animal retrovirus might have been introduced in the homosexual population.

Once this syndrome has become clinically manifest, the various components have been difficult if not impossible to treat, and one may predict that an ultimate mortality rate approaching 70%–80% will be found. It is also apparent that conventional cancer chemotherapy and radiotherapeutic approaches will probably not be tolerable in this population and may not be effective. Where local radiotherapy and single-agent chemotherapy are plausible, an attempt at such treatments should be made, but what is undoubtedly required is a reconstitution of the immune system, again noting that when immunosuppression is withdrawn from renal transplant patients with KS, the lesions may regress. This reconstitution may take the form of marrow transplantation or selective reconstitution with cellular immune elements, but clearly such an approach remains highly experimental and risky. It is also possible that monoclonal antibodies generated against potentially specific KS antigens might be of use therapeutically.

Finally, treatment of the KS and the opportunistic infections is usually unsuccessful at present, and prevention would appear to be the only practical remedy. Since nitrite exposure and promiscuity seem to be the only new elements contributing to this complex and tragic epidemic, both should be discouraged. Even if we cannot identify the contribution of these factors independently, we would soon learn whether such intervention yields a reduction in the number of reported cases.

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Brief Reports

Treatment of Refractory Acute Nonlymphocytic Leukemia With a Combination of Pyrazofurin and Cytarabine[1]

Henry G. Kaplan,* Frederick R. Appelbaum, Martin A. Cheever, Phillip D. Greenberg, Turner Wood, and Alexander Fefer[2]

SUMMARY

Six patients with refractory acute nonlymphocytic leukemia were treated with pyrazofurin (15 mg/m^2) followed by cytarabine (100 mg/m^2) every 12 hours for 6–21 days. All patients cleared their peripheral blood of blast cells but no complete remissions were achieved. Excessive toxicity to skin and mucous membranes was observed. In the doses used, this combination is too toxic for further use. Alternate treatment schedules should be explored.

[Cancer Treat Rep 66:1397–1398, 1982]

Chemotherapy for acute nonlymphocytic leukemia (ANLL) has improved significantly in recent years (1). However, the outlook for patients without matched siblings who fail primary therapy with daunorubicin, cytarabine (ara-C), and 6-thioguanine is still dismal. The introduction of 5-azacitidine (2) and AMSA (3,4) has failed to produce lasting remissions in these patients. In vitro investigations have suggested that the experimental drug pyrazofurin (PZF) may act synergistically with ara-C (5). In this study, we evaluated the activity of maximally tolerable single-agent doses of PZF with ara-C in end-stage patients with ANLL.

METHODS

Six patients with ANLL who had failed either induction or reinduction therapy with daunorubicin or doxorubicin, ara-C, and 6-thioguanine were eligible for study after giving informed consent. Patients were treated with PZF at a dose of 15 mg/m^2 every 12 hours. Two hours after each dose of PZF, patients received ara-C at a dose of 100 mg/m^2. After the first 7 days of therapy, bone marrow aspiration was performed. If the marrow was not aplastic, therapy was continued for another 3–5 days at the same doses and the marrow aspiration was repeated. This regimen was to be continued for a total of up to 21 days of therapy. If, after 21 days of treatment, the bone marrow still contained blast cells, the therapy was considered to be a failure and the patient was removed from the protocol.

Drug therapy was discontinued for toxicity only because of skin reactions severe enough to cause denudation of $\geqslant$ 10% of body surface area or mucositis severe enough to cause denudation of $\geqslant$ 75% of the oropharynx. No patients received diminished therapy because of hematologic toxicity.

RESULTS

All patients experienced complete clearance of blast cells from the peripheral blood and reached nadir absolute granulocyte counts of zero. Granulocyte counts remained < 500/mm^3 for 9–62+ days. Bone marrow examination on Day 8 in the five patients examined demonstrated marked hypocellularity of the normal hematopoietic elements but persistence of blast cells. No patient achieved an M_1 or M_2 marrow. In addition, mucositis and/or skin rash were severe enough to necessitate discontinuation of therapy before the planned 21 days in five of the six patients. In three of these patients, only 1 week of therapy or less was possible. All patients who developed severe skin or mucosal reactions, however, recovered within 3 weeks.

DISCUSSION

Major improvements have been made in the treatment of adult ANLL in recent years. Therapy with combinations including ara-C, 6-thioguanine, and

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[1] Received June 5, 1981; revised Feb 1, 1982; accepted Feb 10, 1982.

[2] Department of Medicine, University of Washington, Seattle.

*Reprint requests to: Henry G. Kaplan, MD, Tumor Institute of the Swedish Hospital Medical Center, 1221 Madison St, Seattle, WA 98104.

daunorubicin now produce complete remissions in upwards of 80% of patients < 50 years old (6). However, therapy for patients who relapse with this combination rarely results in prolonged disease control.

Karaues et al (7) recently reported that treatment of refractory ANLL patients with doses of ara-C in the range of 6 g/m²/day × 6 days produces a complete response rate of 50%, with a median response duration of 5+ months. Such therapy produced prolonged marrow suppression, significant stomatitis, and some cerebellar toxicity. This study suggests that increased uptake of ara-C into leukemia cells would result in clinical responses in patients refractory to standard doses of ara-C.

Another potential method of increasing activation of ara-C would be to inhibit normal de novo cytidine synthesis. Indeed, Cadman et al (5) have demonstrated that PZF, a potent inhibitor of the enzyme orotidylate decarboxylase, produces synergistic antitumor effect against the experimental L5178Y leukemia when PZF precedes ara-C treatment. The use of PZF in combination with ara-C is particularly interesting because Vogler and Trulock (8) have demonstrated that PZF itself possesses significant antileukemia activity in man. Unfortunately, doses > 30 mg/m²/day, which were necessary to obtain this effect, produced unacceptable stomatitis and skin rashes.

Therefore, in this study we utilized 30 mg/m²/day of PZF as pretreatment for ara-C. This combination produced clearing of peripheral blast cells and marrow hypoplasia in all six end-stage patients studied. However, all patients continued to demonstrate some blast cells in their bone marrows and repopulated their peripheral blood with leukemic blast cells without reappearance of normal cells. In addition, five of the six patients experienced extremely severe mucositis and skin rashes necessitating discontinuation of therapy. These results are similar to those reported for the combination of PZF and 5-azacitidine (9).

When used in this way the combination of PZF and ara-C causes unacceptable toxic reactions without producing an obvious improvement in antileukemic effect over currently available regimens. Future studies might explore other dose schedules of these two drugs. In view of the prolonged enzyme inhibition produced by large doses of PZF (5), a single dose of PZF followed by 5–7 days of ara-C would be of particular interest.

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Brief Reports

Non-Cross-Resistant Chemotherapy and Consolidation Radiotherapy for Small Cell Carcinoma of the Lung[1,2]

James A. Young,[3] Robert O. Dillman,[3,*] Stephen L. Seagren,[3] Raymond Taetle,[3] Robert E. Rentschler,[4] James W. Lea, Jr,[5] Thomas J. Lehar,[6] Mark R. Green,[3] William Stanton,[3] John Mendelsohn,[3] and Ivor Royston[3]

SUMMARY

Fifty-six patients with small cell carcinoma of the lung were treated with a two-cycle induction course of hexamethylmelamine, vincristine, doxorubicin, and cyclophosphamide. Patients with limited disease (LD) who responded and patients with extensive disease (ED) who had a complete response received prophylactic whole-brain radiotherapy, as well as radiotherapy to thoracic and abdominal sites of disease. Concurrently with radiotherapy, consolidation chemotherapy was given with doxorubicin, cyclophosphamide, methotrexate, and etoposide. The complete response rate was 35% for ED patients and 68% for LD patients. The median survival time for complete responders was 54 weeks for ED patients and 65 weeks for LD patients. The toxicity of the program was moderate, and the effectiveness was comparable to that of other reported combined-modality treatment programs.

[Cancer Treat Rep 66:1399–1401, 1982]

Aggressive combination chemotherapy for small cell carcinoma of the lung (SCCL) can yield complete responses, and a number of groups have employed combined-modality therapy with the goal of effecting long-term survival and cure in this disease (1–3). The treatment program described here was developed by the San Diego Oncology Group to incorporate new chemotherapy agents and scheduling techniques, together with consolidative radiotherapy, in an effort to improve treatment results in SCCL.

PATIENTS AND METHODS

Patients with histologically documented SCCL and a Karnofsky performance status of at least 20 were eligible for the study. On the basis of initial staging studies, patients were designated as having limited disease (LD) if their involvement was confined to one hemithorax, the mediastinum, and ipsilateral supraclavicular lymph nodes. All other patients were designated as having extensive disease (ED).

Induction chemotherapy (HexaVAC) was given in two 28-day cycles as follows: 100 mg/m^2 of hexamethylmelamine orally on Days 2–15, 1.5 mg/m^2 of vincristine (maximum, 2.0 mg) iv on Days 1 and 8, 500 mg/m^2 of cyclophosphamide iv on Days 1 and 8, and 25 mg/m^2 of doxorubicin iv on Days 1 and 8. After two cycles of HexaVAC, six alternating 28-day chemotherapy cycles (CMV/AMV) were administered as follows: 1.0 g/m^2 of cyclophosphamide on Day 1, 200 mg/m^2 of etoposide iv on Day 1, and 30 mg/m^2 of methotrexate iv on Day 21 (CMV), followed by 50 mg/m^2 of doxorubicin iv on Day 1, 200 mg/m^2 of etoposide iv on Day 1, and 30 mg/m^2 of methotrexate iv on Day 21 (AMV).

Radiotherapy was given to all LD patients who achieved a complete response (CR) or partial response (PR) with induction chemotherapy and to ED patients who achieved a CR during induction chemotherapy. Treatment began concurrently with the first CMV cycle. Prophylactic whole-brain radiation was given as 2000 rad in ten fractions over 2 weeks. Therapy to the chest was given in two

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[1] Received July 1, 1981; revised Feb 3, 1982; accepted Feb 16, 1982.

[2] Presented at the American Society of Clinical Oncology Meetings, Washington, DC, April 29, 1981.

[3] Divisions of Hematology/Oncology (*J. A. Young, R. O. Dillman, R. Taetle, M. R. Green, W. Stanton, J. Mendelsohn,* and *I. Royston*) and Radiation Oncology (*S. L. Seagren*), University of California, San Diego.

[4] Loma Linda University Medical Center, CA.

[5] San Diego Naval Regional Medical Center, CA.

[6] Rees-Stealy Clinic, San Diego, CA.

**Reprint requests to:* Robert O. Dillman, MD, Veterans Administration Medical Center, 3350 La Jolla Village Dr, V-111 E, San Diego, CA 92161.

TABLE 1.—Response rates and survival data by stage of disease and degree of response

Disease stage	No. of patients	% response		Median survival (wks)		
		CR	PR	CR	< CR	Overall
ED	34	35	26	54	39	42
LD	22	68	14	65	36	60
All patients	56	48	21	61	38	50

courses, each consisting of 2000 rad in ten fractions over 2 weeks. The second half of radiotherapy was given during the second CMV cycle, after a break of approximately 6 weeks. The thoracic radiotherapy treatment volume encompassed the entire pretreatment area of the primary tumor and included the mediastinal and ipsilateral hilar nodes. In addition to thoracic radiotherapy, ED patients who attained a CR while receiving induction chemotherapy were given 2000 rad of consolidative irradiation to areas of known intra-abdominal disease.

All patients entered in the study and receiving any therapy were considered to be evaluable. CR was defined as the total disappearance of all evidence of disease; however, CR was not documented by bronchoscopy in most cases. PR was defined as a > 50% reduction in the product of the two largest perpendicular diameters of measurable disease. The assessment of whether chest relapse occurred within irradiated fields was made by direct comparison of port films with serial chest X-rays. Projected median survival was calculated using the technique of Kaplan and Meier, and differences in survival were assessed using the Mantel-Haenszel method.

RESULTS

Between July 1978 and October 1980, a total of 56 patients, 45 men and 11 women, were entered in the study. The median age was 60 years (range, 43–81), and the median Karnofsky performance status was 90 for LD patients and 70 for ED patients. The median followup at the time of analysis was 85 weeks.

The response rate and survival data are presented in table 1. Overall, 69% of the patients had at least a PR and 48% of the patients had a CR. The CR rate was 68% for LD patients and 35% for ED patients. The median survival time was 42 weeks for ED patients and 60 weeks for LD patients ($P = 0.006$). Complete responders in the ED group had a median survival of 54 weeks, and those in the LD group had an estimated median survival of 65 weeks. Of the ED patients, only two are alive and free of disease (40+ and 65+ weeks). In the LD group, ten patients are alive and free of disease, with five beyond 1 year (62+, 76+, 88+, 109+, and 124+ weeks). In both groups, survival for patients achieving a CR was significantly better than for those who did not ($P < 0.01$). Of the CRs, 44% occurred during induction chemotherapy and 56% occurred during the consolidation phase of treatment. The survival of patients in the early CR and late CR groups was not significantly different.

Of the 29 patients who received protocol thoracic radiotherapy, ten (40%) had disease recurrence within the radiotherapy field. Two ED patients were given hepatic irradiation for consolidation after attaining CR. Both patients had pronounced thrombocytopenia after treatment, and both had disease recurrence in the liver. Of 37 patients receiving prophylactic whole-brain radiotherapy, three (8%) had brain recurrence documented by computerized tomographic scan.

Severe leukopenia (wbc count < 2000/mm^3) occurred in 21% of patients during HexaVAC chemotherapy and in 16% of patients during the CMV/AMV phase of therapy. All other toxic reactions were graded as severe or life-threatening in < 10% of the cases. Five deaths, all in ED patients, occurred within the first 8 weeks of therapy. Two patients died from sepsis during treatment-induced leukopenia, one patient had an apparent myocardial infarction, and two patients died with progressive tumor. Toxicity from concurrent radiotherapy and CMV/AMV chemotherapy was moderate and manageable. Two patients had transient cessation of all oral intake due to esophagitis, and one patient required hospitalization for radiation pneumonitis. Chemotherapy dose reduction or delay was required for 11% of the HexaVAC cycles, 5% of the CMV/AMV cycles given without concurrent radiotherapy, and 31% of the CMV/AMV cycles given with concurrent radiotherapy.

DISCUSSION

Hexamethylmelamine, an investigational agent with a response rate of 43% as a single agent in SCCL (4), was added to an effective and established three-drug combination regimen (CAV) to form a four-drug induction regimen (HexaVAC). This combination was well-tolerated and yielded, after only two cycles, a 36% CR rate in the LD patients and a 12% CR rate in the ED patients. When compared to response rates achieved by other groups using CAV alone (5,6), these data are encouraging and may warrant further study of the HexaVAC combination in randomized trials. After induction chemotherapy, non-cross-resistant chemotherapy was prescribed in an attempt to increase the CR rate. Etoposide, an investigational agent with 45% activity in SCCL (7), and methotrexate were added in the CMV/AMV consolidation chemotherapy. Although dose reduction or delay was required in a significant number of chemotherapy cycles given with concurrent radiotherapy, over one half of the CRs in the present study occurred during this phase of treatment.

Thoracic radiotherapy was used to achieve further cytoreduction at bulky sites of disease. Split-course radiotherapy was used to minimize the local toxic reactions associated with standard scheduling and to permit uninterrupted chemotherapy. This study demonstrated the feasibility and patient tolerance of such scheduling. A 40% relapse rate within the irradiated volume occurred in spite of the fact that thoracic radiotherapy was given only to patients achieving significant reduction in tumor bulk with induction chemotherapy. This corroborates the observation of other investigators that thoracic radiotherapy, given in several different doses and fractionation schedules, has been unsuccessful in preventing local relapse in SCCL (8,9). Only 8% (< 20% with 95% confidence) of patients receiving 2000 rad of prophylactic whole-brain radiotherapy had brain relapse. This relapse rate is comparable to that observed in other series employing higher doses (1,2).

Long-term survival in SCCL continues to be an elusive goal, and in most series < 15% of LD patients survive > 2 years (1–3). The present treatment program was reasonably successful in inducing CR, but most of these patients eventually relapsed and died from recurrent, drug-resistant neoplasm. On balance, the results of the present study are comparable to the best reported series of combined-modality therapy for SCCL.

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Brief Reports

Cisplatin, Cyclophosphamide, and Prednisone Therapy for Stage D Prostatic Cancer[1]

J. Berry* and R. N. MacDonald[2]

Stage D prostatic cancer has a variable natural history with a median patient survival of 1–3 years (1). The objective response to chemotherapy is difficult to document (2–4). Both cisplatin and cyclophosphamide as single agents have demonstrated some activity in the management of prostatic cancer (5,6). Prednisone was selected to act on those cancer cells that might remain hormonally sensitive.

PATIENTS AND METHODS

This phase III study was initiated in March 1977 in patients with hormonally resistant stage D prostatic cancer. Patients were given cyclophosphamide (150 mg/day) for 14 days orally, cisplatin (50 mg/m^2) iv on Day 8, and prednisone (5 mg) orally three times daily, continuously throughout the 28-day cycle. Before and after hydration, steps were taken to minimize the risk of cisplatin-induced nephrotoxicity; the dose was reduced by 50% in the presence of a reduced creatinine clearance (< 50 ml/minute), and cisplatin administration was discontinued when the clearance was < 50% of pretreatment values.

The dose of cyclophosphamide was modified if the segmented neutrophil count was < 2000/mm^3 or if the platelet count was < 100,000/mm^3. Termination of treatment occurred when objective progression of disease was evident after two full cycles of therapy.

Patient Characteristics

Twenty-two patients were entered in the study until December 1980. The median age was 64 years (range, 51–80). All had received prior hormonal therapy in the form of orchiectomy, diethylstilbestrol, and/or diethylstilbestrol diphosphate. Eleven patients had received irradiation previously. Sites of disease were bone in 22 patients and lymph nodes in five; none of the patients had visceral disease. Performance status (PS) was assessed according to the Eastern Cooperative Oncology Group criteria; one patient had PS 0; eight, PS 1; three, PS 2; five, PS 3; and five, PS 4.

RESULTS AND DISCUSSION

Responses were defined in accordance with the criteria set by the National Cancer Project. No objective or partial responses were documented in the 22 patients; ten (45%) achieved stable disease status and one had subjective improvement despite progressive disease. The overall median survival was 9 months; median survival was 10 months in patients with stable disease and 5 months in patients with disease progression. The median duration of response was 6 months. No statistically significant difference in survival was demonstrated between responders and nonresponders. Three of five patients with measurable nodes had a > 50% reduction in their size but failed to have a corresponding decrease in acid phosphatase to normal levels, required to define a partial remission. Seven patients in the objectively stable group had a > 50% reduction in serum acid phosphatase, and three had a return of alkaline phosphatase levels to normal. Subjective improvement as defined by decreased pain, improvement in performance status, and weight gain was seen in the ten objectively stable patients as well as in one patient who had disease progression by National Cancer Project criteria. The median number of cycles of therapy was four (range, 1–14). Toxicity was within acceptable limits, considering the number of patients who had received prior irradiation and who had extensive bone marrow involvement. Only three patients experienced significant neutropenia with segmented neutrophil counts < 1000/mm^3. Two of these three had coexistent thrombocytopenia of < 100,000 but > 20,000/mm^3. There was no evidence of disseminated intravascular coagulation in these patients.

Despite the age and poor general status of many patients, cisplatin was reasonably well-tolerated. Only three patients had initial reduction in cisplatin dose due to low pretreatment creatinine clearances.

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[1] Received Aug 17, 1981; revised Jan 25, 1982; accepted Jan 29, 1982.

[2] Southern Alberta Cancer Centre, Provincial Cancer Hospitals Board, Calgary, Canada.

Reprint requests to: Dr. John Berry, Southern Alberta Cancer Centre, Provincial Cancer Hospitals Board, 1331–29th St. NW, Calgary, Alberta, T2N 4N2, Canada.

There was no significant deterioration in creatinine clearance values after discontinuation of cisplatin in the face of advancing disease. Eight patients experienced moderate to severe nausea, with only four having moderate to severe vomiting (more than four episodes within a 24-hour period). One patient developed ototoxic effects secondary to cisplatin.

In this small unselected group, our results did not show any superiority to those achieved by single-agent therapy; approximately 50% of the patients experienced improved pain control with diminished analgesic requirements and improved performance status. In contrast to other studies, the objectively stable patients (45%) did not gain a significant survival benefit. The addition of prednisone to the regimen may have interfered with analysis by improving appetite and sense of well-being. Whether the administration of prednisone altered the androgenic pathway in addition to the expected glucocorticoid suppression is not known, because hormonal parameters were not measured during the course of treatment. It became apparent that unless significant pain relief occurred after one cycle of treatment, further therapy would fail. Most treatment, with the exception of the initial course, was done on an outpatient basis.

The initial response to hormonal maneuvers in stage D prostatic cancer is usually about 80%. However, because the disease will ultimately progress and because some patients are hormonally resistant to initial treatment, there is a need to develop more effective chemotherapeutic regimens. The combination of cisplatin, cyclophosphamide, and prednisone in our group of patients provided good short-term palliation with acceptable toxicity, although it did not show any clear-cut benefit over other combinations or over single-agent therapy currently used.

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VAB-3 Combination Chemotherapy in Primary Mediastinal Germ Cell Tumors[1,2]

Davor Vugrin,* Nael Martini, Willet F. Whitmore, Jr, and Robert B. Golbey[3]

SUMMARY

Ten patients with primary mediastinal nonseminomatous germ cell tumors were treated with the VAB-3 protocol. All patients with measurable disease showed tumor regression with chemotherapy, but only two had sustained partial response allowing thoracotomy and resection of residual tumor after the second induction. Both patients received additional induction therapy and one also received irradiation; they have been free of disease 41+ and 40+ months from the beginning of chemotherapy. All other patients died from the disease.

[Cancer Treat Rep 66:1405–1407, 1982]

Primary germ cell tumors of the mediastinum account for 1%–4% of tumors arising in the mediastinum (1,2) and are histologically identical to those arising in the testis. Distinction between seminomatous and nonseminomatous tumors determines the prognosis and treatment. Seminoma accounts for about 30% of all primary mediastinal germ cell tumors, and surgery alone or combined with irradiation results in apparent cure in about 50% of patients (2–6). The results with nonseminomatous germ cell tumors are poor (3,7). This is a review of ten patients with primary mediastinal germ cell tumors treated with VAB-3 combination chemotherapy.

MATERIALS AND METHODS

Between September 1975 and February 1978, ten male patients with apparent primary mediastinal germ cell tumors were entered in the VAB-3 protocol at the Memorial Sloan-Kettering Cancer Center (MSKCC). Eight of these patients were white and two were black. Nine patients had nonseminomatous germ cell tumors and one had a biopsy-proven seminoma but elevated serum alpha-fetoprotein and beta-human chorionic gonadotropin, indicating the presence of nonseminomatous elements. The diagnosis was established in all patients by thoracotomy or mediastinotomy and biopsy. Histologic diagnosis was made by the Pathology Department of MSKCC. Two patients had received mediastinal irradiation and one other had received chemotherapy prior to referral to MSKCC. Nine patients had measurable disease that was bulky in eight and nonbulky (< 5 cm in diameter) in one. One patient had complete resection of all gross disease and no measurable tumor at the beginning of chemotherapy.

To assess extent of disease prior to chemotherapy, each patient had a workup that included physical examination, chest X-ray, bone scan, liver and spleen scans if liver function was abnormal, and neurologic computerized tomographic scan, when indicated. A number of patients had pedal lymphangiogram and iv pyelogram.

The VAB-3 regimen consisted of two hospital inductions of 8 days each, 5–6 months apart, and outpatient treatments every 3 weeks (table 1). Prior to each induction, each patient had a pulmonary function test and a 24-hour creatinine clearance.

Responses were defined as complete and partial. Complete response was defined as the elimination of all evidence of disease for a minimum of 3 months. All other patients were partial responders. There was no dose modification for chemotherapy. If the leukocyte count was < 3800/mm³ or the platelet count < 150,000/mm³, chemotherapy was delayed until recovery.

Received Sept 1, 1981; revised Dec 15, 1981; accepted Jan 5, 1982.

Supported in part by Public Health Service grant CA-05826 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Brian Piccolo Memorial Fund.

Solid Tumor Service, Department of Medicine (*D. Vugrin* and *R. B. Golbey*); Thoracic Service, Department of Surgery (*N. Martini*); and Urology Service, Department of Surgery (*W. F. Whitmore, Jr*); Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Davor Vugrin, MD, Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

TABLE 1.—VAB-3 schedule

Day or treatment No.	Drug	Dose and route
	Induction	
Day 1	Cyclophosphamide	600 mg/m^2 iv
	Vinblastine	4 mg/m^2 iv
	Dactinomycin	1 mg/m^2 iv
	Bleomycin	30 mg iv
Days 1–7	Bleomycin	20 mg/m^2/day by continuous infusion for 7 days
Day 8	Cisplatin	120 mg/m^2 iv with mannitol-induced diuresis
	Consolidation 3 wks later: treatment every 3 wks × 6	
Treatment 1 and 4	Vinblastine	4 mg/m^2 iv
	Doxorubicin	30 mg/m^2 iv
	Chlorambucil	4 mg/m^2 orally daily for 14 days
Treatment 2 and 5	Vinblastine	4 mg/m^2 iv
	Dactinomycin	1 mg/m^2 iv
	Chlorambucil	4 mg/m^2 orally daily for 14 days
Treatment 3 and 6	Vinblastine	4 mg/m^2 iv
	Cisplatin	50 mg/m^2 iv
	Chlorambucil	4 mg/m^2 orally daily for 14 days
	Reinduction 3 wks later: same as induction	
	Maintenance 3 wks later: treatment every 3 wks for 18 mos	
	Vinblastine	4 mg/m^2 iv
	Dactinomycin	1 mg/m^2 iv
	Chlorambucil	4 mg/m^2 orally daily for 14 days

RESULTS

Ten patients received the initial VAB-3 induction therapy and all nine with measurable disease showed regression of tumor. In seven patients (including one without measurable disease at the start of chemotherapy), however, the disease progressed before the scheduled second induction 5–6 months later. Three patients with sustained partial response to the first induction received a second: one subsequently progressed and two had thoracotomy approximately 8 weeks after the last induction. In one patient microscopic foci of embryonal carcinoma were resected from the pericardium and he received an additional two inductions; he remains free of disease 40+ months from the beginning of chemotherapy. The other patient who had bone metastases at the beginning of treatment had 99% resection of the primary tumor, irradiation to the humerus, two further inductions, and irradiation to the mediastinum; he is free of disease 41+ months from the beginning of chemotherapy and 36+ months from the last surgery.

One patient had a temporary increase in serum creatinine (> 2 mg/100 ml) after a second induction with cisplatin. One patient was treated with broad-spectrum antibiotics when he developed fever during myelosuppression.

DISCUSSION

Effective chemotherapy appears essential for the control of primary mediastinal nonseminomatous germ cell tumors but the rarity of this tumor and the recent evolution of effective chemotherapy have limited accumulation of relevant experience. Current policy is to treat patients with primary mediastinal nonseminomatous germ cell tumors with chemotherapy regimens identical to those used for histologically similar germ cell tumors of the testis. During the past decade, combination chemotherapy, variously incorporating cisplatin, bleomycin, vinblastine, and other agents, has improved results in the treatment of disseminated testis cancer. Currently, the potential for cure of patients with advanced germ cell tumors of the testis is approximately 80% (8).

The results presented here for the treatment of primary mediastinal germ cell tumors are worse than those reported for treatment of testis cancer with the VAB-3 regimen, probably because patients with primary mediastinal germ cell tumors have a greater bulk of disease at the beginning of chemotherapy (9). It is our impression that patients with primary mediastinal germ cell tumors have a response to chemotherapy alone which is similar to t at of patients with equally bulky germ cell tumors o the testis.

A sustained response to chemotherapy was cruc l for complete remission, but both patients a hieving such a response required additional focal t atment with surgery and/or irradiation to achieve c mplete remission. All patients who showed tumor p ogression in spite of chemotherapy died.

Despite recent optimism in the treatment of pati nts with nonseminomatous primary mediastinal g m cell tumors, cures are still the exception rather t an the rule. From experience with primary germ ce l testis tumors supplemented by this small expe rience with mediastinal germ cell tumors, one may tentatively conclude that patients with primary mediastinal nonseminomatous germ cell tumors should be treated initially with chemotherapy employing successive inductions at shorter intervals (ie, VAB-6 protocol) (8). This should be followed by an attempt at surgical resection of residual disease. When the resected tissue shows malignant elements, additional inductions are recommended.

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Vindesine (VDS) Monochemotherapy for Non-Small Cell Lung Cancer: A Report of 45 Cases[1,2]

Brief Reports

Susan L. Luedke,* Dan W. Luedke, Paul Petruska, G. O. Broun, Garry Reed, and John Leavitt[3,4]

SUMMARY

Fifty-three patients with non-small cell lung cancer were treated with vindesine. Of the 45 evaluable patients, 11 (24%) had a partial response. Responses were all evident within 6 weeks. Median duration of response was 8 weeks from documentation. Median survival was not improved significantly among responders ($P < 0.10$, two-tailed test of significance). The most frequent toxic effect was leukopenia. The most troublesome toxic effect was peripheral neuropathy, with patients > 60 years old experiencing this more frequently.

[Cancer Treat Rep 66:1409–1411, 1982]

In 1979, Gralla et al (1) reported the results of vindesine (VDS) treatment of metastatic non-small cell lung cancer (NSCLC). Ten of 46 evaluable patients had a partial response (PR). In light of this promising report, we initiated trials of VDS in patients with advanced epidermoid carcinoma and adenocarcinoma of the lung.

MATERIALS AND METHODS

Patients were eligible for treatment if they had pathologically proven NSCLC that was incurable and measurable; had normal renal, hepatic, and bone marrow function (unless tumor-related abnormalities were present); had an expected survival of at least 6 weeks; and had given written informed consent.

VDS was administered at a dose of 3 mg/m^2 (increased to 4 mg/m^2) by iv bolus injection once weekly for 10 weeks, followed by 3 mg/m^2 biweekly. Dose attenuation was made on the basis of hematologic and neurologic toxicity as previously described by Gralla et al (1). Patients were considered evaluable for response if they were in the study 6 weeks (or less with documented disease progression). Response to therapy was defined as follows: *PR*—a ≥ 50% reduction in tumor size; *minor response*—a < 50% but ≥ 25% reduction in tumor size; *stable disease*—neither response nor disease progression for at least 6 weeks; and *progressive disease*—a ≥ 25% increase in tumor size or appearance of new disease outside the central nervous system. Duration of response was measured from the time of documentation, and response had to be present for a minimum of 1 month.

RESULTS AND DISCUSSION

Fifty-three patients were treated with VDS, with 85% fully evaluable. Twenty-eight patients with epidermoid carcinoma were part of a protocol comparing VDS with cyclophosphamide and doxorubicin; they will also be reported on elsewhere. Of the eight inevaluable patients, seven died within the first 3 weeks; death was unrelated to therapy and there was no measurable change in disease. The remaining patient had an erroneous pathology diagnosis.

Patient characteristics in relation to response are shown in table 1. Weight loss, extensive disease [by Veterans Administration Lung Group criteria (2)], and prior radiation therapy were associated with poor response to VDS. Eleven (24%) of the patients had a PR with a median duration of 8 weeks. An additional four patients had a minor response. This response rate (24%) is remarkably similar to the 21% reported by Furnas et al (3) in their compilation of 146 patients with NSCLC treated with VDS monotherapy. Response was evident within the first 6 weeks of therapy. Sites of response were the pri-

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[1] Received Sept 17, 1981; revised Dec 29, 1981; accepted Jan 20, 1982.

[2] Supported in part by a grant from Eli Lilly Co.

[3] Division of Hematology/Oncology, St. Louis University School of Medicine and St. Louis Veterans Administration Medical Center, MO.

[4] We thank Jean Schlueter, Linda Hackney, and Angelia Estes for their assistance.

* *Reprint requests to:* Susan L. Luedke, MD, St. Louis University Medical Center, School of Medicine, 1325 S Grand Blvd, St. Louis, MO 63104.

mary lesion and lymph nodes (bone metastases were not considered measurable). Symptomatic brain metastases developed while patients were responding peripherally. Otherwise, relapse occurred principally in sites of measurable disease. Median survival was 14 weeks (fig 1) with no significant difference in survival between patients responding and those with stable or progressive disease (P < 0.10). This must be interpreted with caution a the treated patient population was heterogeneou in prognostic characteristics.

Toxic effects due to VDS were principally ne tropenia and neurotoxicity. Neutropenia was th most frequent dose-limiting toxic reaction (table 2

TABLE 1.—Patient characteristics and response to VDS chemotherapy

Characteristic	Total No. of patients (%)	No. of patients (%) with— PR	PR + MR*
Karnofsky performance status			
80–100	36 (80)	10 (28)	14 (39)
≥ 60, < 80	5 (11)	0	0
≤ 50	4 (9)	1	1
Epidermoid carcinoma	35 (78)	9 (26)	13 (37)
Adenocarcinoma	10 (22)	2 (20)	2 (20)
Extensive disease	34 (76)	3 (9)	6 (18)
Weight loss > 10%	19 (44)	2 (10)	4 (22)
Sex			
Male	39	10	14
Female	6	1	1
Prior radiotherapy	17 (38)	2 (12)	2 (12)
Prior chemotherapy	6 (13)	1 (17)	2 (33)

*MR = minor response.

TABLE 2.—VDS hematologic toxicity

Toxicity	No. of patients (%)
Wbc count (cells/mm^3)	
≥ 4000	11 (24)
< 4000, ≥ 2500	17 (38)
< 2500	17 (38)
Platelet count (cells/mm^3)	
> 450,000	14 (31)
< 150,000, ≥ 100,000	12 (27)
< 100,000, ≥ 50,000	1
< 50,000	1

TABLE 3.—VDS neurotoxicity

Characteristic	Total No. of patients	No. of patients with neurotoxicity (%)	Significance (chi-square)
Age in yrs			
> 60	26	10 (38)	$P < 0.05$
≤ 60	19	13 (68)	
Total VDS (mg/m^2)			
> 18	24	15 (63)	$P < 0.10$
≤ 18	21	8 (38)	
Evaluable	45	23 (51)	

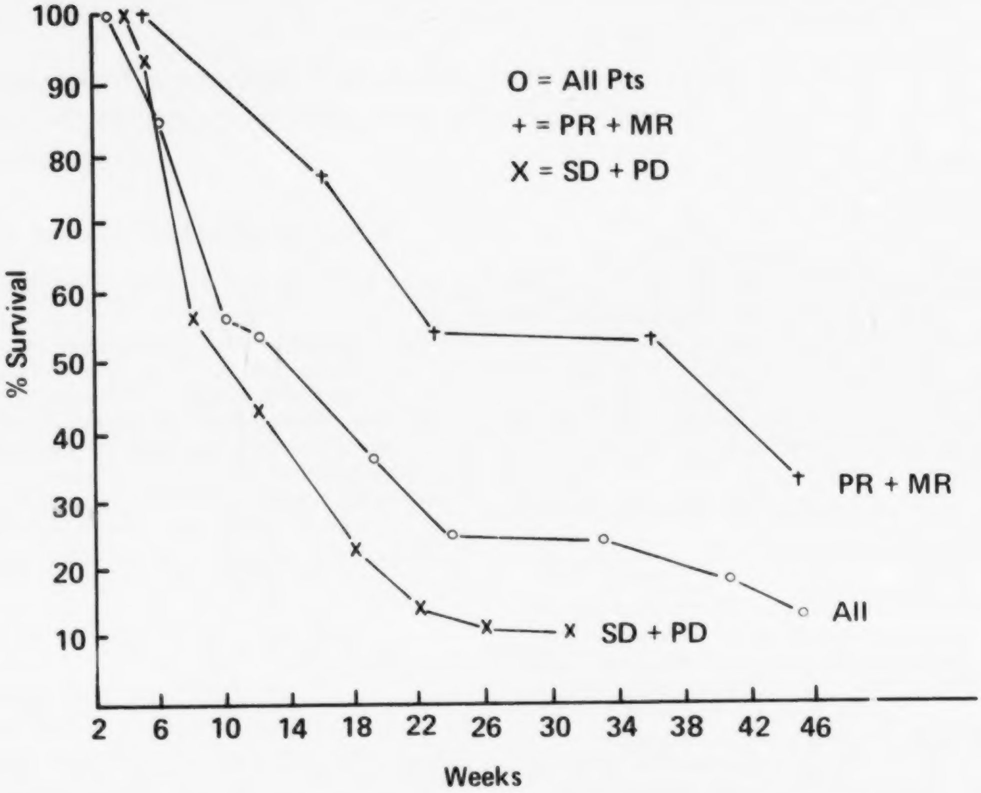

FIGURE 1.—Survival of all patients with NSCLC treated with VDS compared to those with PR or minor response (MR) and those with stable disease (SD) or progressive disease (PD).

No life-threatening infection or bleeding episode was found as a result of bone marrow suppression. Peripheral neuropathy was the most troublesome toxic effect (table 3), with patients > 60 years of age experiencing this more frequently ($P < 0.05$). Although dose-dependent neuropathy with VDS has been reported elsewhere, this age-dependent neuropathy has not been described in other VDS clinical studies nor in two recent reviews of vincristine neurotoxicity (4–6).

We conclude that VDS is an active drug in NSCLC. Dose increase above 3 mg/m^2 does not appear to be warranted.

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Brief Reports

Treatment of Advanced Plasma Cell Myeloma With Weekly Cyclophosphamide and Alternate-Day Prednisone[1]

Lorne J. Brandes* and Lyonel G. Israels[2,3]

SUMMARY

Three of five patients with refractory myeloma were successfully palliated using a simple and relatively nontoxic regimen consisting of weekly cyclophosphamide in combination with alternate-day oral prednisone. Control of advanced disease in some patients may be associated with more frequent scheduling of known active drugs, even though these drugs, either alone or in combination, may not be effective when given at standard intervals of 4–6 weeks.

[Cancer Treat Rep 66:1413–1415, 1982]

Approximately 50% of patients with multiple myeloma achieve remission when treated with either melphalan or cyclophosphamide as single agents in association with prednisone (1). With the possible exception of a five-drug combination (2), the remission rate and survival in this disease have improved only marginally with the use of more aggressive combination chemotherapy, either in alternating or sequential schedules (3–5).

Regardless of the type of induction therapy, remission is achieved in most cases with < 2-log cell kill (6). A plateau phase follows where, despite continuing cyclic therapy, a further reduction in M protein does not occur (7). Eventually, an accelerated phase of the disease, characterized by an increase in labeling index, occurs (8). Treatment of patients in this malignant phase has tended to be more aggressive, but despite the use of multiple-drug regimens, overall survival has been poor and drug toxicity has been high (9).

We report the palliative treatment of five patients with advanced myeloma, using a simple and relatively nontoxic regimen consisting of weekly cyclophosphamide (150–300 mg/m^2 iv) in combination with alternate-day oral prednisone (50–100 mg).

MATERIALS AND METHODS

Patients

Five patients with advanced myeloma who were either initially or subsequently refractory to melphalan and prednisone and then to multiple-drug regimens were treated with weekly cyclophosphamide and alternate-day prednisone.

The treatment regimen consisted of alternate-day oral prednisone (50–100 mg) and weekly low-dose iv cyclophosphamide (150–300 mg/m^2; maximum weekly dose, 500 mg). Because a 5-month switch to oral weekly cyclophosphamide from the iv route in patient 1 resulted in disease progression which was corrected within 2 months of reinstitution of the iv schedule, all subsequent doses of cyclophosphamide in this patient and in the subsequent patients were given iv.

Patients were considered evaluable after having completed at least eight weekly injections of cyclophosphamide.

Response to therapy was defined as a sustained decrease in M protein (< 50% of the pretreatment value), a significant improvement in anemia or pancytopenia, correction of hypercalcemia where this condition existed, and stabilization of lytic bone lesions.

RESULTS

The results of therapy are shown in table 1. Three of five patients (Nos. 1–3), each with a serious complication of this disease, responded well to therapy. Patients 4 and 5 failed to respond and died within 2 months of starting weekly cyclophosphamide and

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[1] Received Sept 21, 1981; revised Dec 22, 1981; accepted Jan 12, 1982.

[2] Department of Medicine, University of Manitoba and the Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada.

[3] We thank Drs. J. Lezack and P. Galbraith for referring patients.

**Reprint requests to*: L. J. Brandes, MD, Department of Medicine, University of Manitoba, Manitoba Institute of Cell Biology, 100 Olivia St, Winnipeg, Manitoba, Canada R3E OV9.

TABLE 1.—Patient characteristics and response to therapy*

Patient No.	Sex/age (yrs)	Type	Previous treatment	M protein level (serum = g/dl; urine = g/24 hrs)		Hemoglobin (g/dl)		Complication(s) at time of initiation of CP	Result of CP therapy	Duration of response†
				Before CP	After CP	Before CP	After CP			
1	M/52	IgG κ	MP (2½ yrs); BAP (13 mos); C × 1 course (1 g over 4 days; failed to respond)	1.6 g/dl	0.6 mg/dl	7	12	Leukemic phase (plasma cells = 9000/μl); pancytopenia	Resolution of pancytopenia and plasmacytosis	24 mos‡
2	F/52	IgA λ	MP (8 yrs); VBAP q4wks × 7 courses + radiotherapy (failed to respond)	3.0 g/dl	0.8 g/dl	9	12	Hypercalcemia	Resolution of hypercalcemia	14+ mos
3	F/68	IgG κ	MP × 3 courses; VMCP q4wks × 3 courses (failed to respond)	5.6 g/24 hrs	0.7 g/24 hrs	7	12.5	Myeloma kidney; creatinine clearance (< 5 ml/min); pancytopenia	Increase in creatinine clearance (39 ml/min); normalization of Hgb and platelets; wbc remains low (2.5×10^3 cells/mm^3)	12+ mos
4	F/72	Light chain disease	MP (18 mos); VBAP × 3 courses; VCMP × 3 courses (failed to respond)	2.0 g/24 hrs	Not quantitated	7	7	Hypercalcemia	Failed to respond (10 courses)	—
5	M/66	IgG λ	MP × 6 courses VBAP × 2 courses; VMCP × 3 courses; (failed to respond)	3.5 g/dl	5.0 g/dl	8	7	Pancytopenia	Failed to respond (8 courses); hypercalcemia	—

*M = melphalan; P = prednisone; V = vincristine; B = BCNU (carmustine); A = doxorubicin; CP = weekly cyclophosphamide and alternate-day prednisone.

†Duration of response is calculated from date CP started.

‡Patient died from septicemia while still responding to CP.

alternate-day prednisone. The responding patients had a prolonged survival, with patients 2 and 3 still receiving therapy at 14+ and 12+ months, respectively. After 24 months of receiving treatment, and while still responding, patient 1 suddenly developed an overwhelming septicemia and died; *Listeria monocytogenes* was cultured from his blood and spinal fluid.

Toxic effects associated with this therapy were limited to one episode of herpes zoster in patient 3 which resolved within 2 weeks. Nausea and vomiting were minimal to nonexistent in all patients; alopecia was not seen. Patients 1 and 3 had disease-associated pancytopenia that subsequently improved with therapy.

DISCUSSION

Myeloma resistant to melphalan has a poor prognosis. Recent reports of response to aggressive multidrug therapy in advanced myeloma have been disappointing. The Southeastern Cancer Study Group reported a 13% response rate in patients treated at 4-week intervals with a combination of BCNU, cyclophosphamide, and prednisone (BCP) after initial failure of melphalan and prednisone therapy (10). In a similar trial, Kyle et al (11) reported an objective response rate of 17% in 42 melphalan-resistant patients treated with BCP every 6 weeks and of only 7% in 47 similar patients treated with cyclophosphamide and prednisone every 6 weeks.

A five-drug protocol (M2) consisting of BCNU, cyclophosphamide, melphalan, vincristine, and prednisone given in 5-week cycles has produced good responses in 13 of 26 patients who were previous treatment failures (2). However, only one of five patients who stopped therapy and entered the aggressive phase of the disease responded to reinstitution of this regimen. In addition, toxicity associated with this treatment included nausea, vomiting, leukopenia, infection, and hemorrhage (2).

Houwen et al (12) have reported the successful palliation of six of 11 patients with advanced myeloma who were treated with a combination of prednisone and vindesine. In this study the vindesine was given weekly for three injections followed by a 3-week rest interval and then repeated.

We suggest that success in controlling advanced disease in some patients may be achieved by the more frequent scheduling of known active drugs, even though the same drugs, either alone or in combination, may not be effective when given at standard monthly intervals. This may also account for the discrepancy between an earlier study of Bergsagel et al (13) demonstrating that cyclophosphamide benefited six of 19 patients with melphalan-resistant myeloma when given at a dose of 1 g/m^2 every 3 weeks, and that of the same investigator in the National Cancer Institute of Canada study which showed only a 5% response rate in melphalan-resistant patients when cyclophosphamide was given in a higher dose at 4-week intervals (4). Similarly, the Southwest Oncology Group has noted the highest response rates in schedules with the shortest treatment intervals (3). The response rate of 55% achieved by Houwen et al (12) to vindesine at weekly intervals is consistent with this concept.

The successful palliation of three of five patients with advanced myeloma using a simple regimen is of clinical importance. Moreover, the three responding patients had serious complications of their disease and in each case control was achieved with little or no associated drug toxicity. The National Cancer Institute of Canada is currently evaluating this approach in the treatment of patients with advanced myeloma who are refractory to melphalan and prednisone.

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Brief Reports

Phase II Study of Cisplatin, Maytansine, and Chlorozotocin in Small Cell Lung Carcinoma (EST 2578)[1,2]

Richard H. Creech,* Kenneth Stanley, Steven E. Vogl, David S. Ettinger, Philip D. Bonomi, and Omar Salazar[2,3]

SUMMARY

Seventy-three patients with small cell lung carcinoma refractory to standard chemotherapy were entered in this phase II randomized study of cisplatin, maytansine, and chlorozotocin. Of the 58 evaluable patients, only one partial response was observed among 21 patients given cisplatin, and no responses were seen among 19 given maytansine or 18 given chlorozotocin. One patient treated with chlorozotocin and two treated with cisplatin experienced life-threatening thrombocytopenia. One third of the maytansine-treated patients experienced moderate or severe neurologic toxicity. The overall median survival was 9.7 weeks. Chlorozotocin treatment was associated with inferior survival (7.7 weeks).

[Cancer Treat Rep 66:1417–1419, 1982]

In this phase II study of small cell lung carcinoma refractory to standard chemotherapy, patients were randomized to receive one of three chemotherapeutic agents that had not previously received adequate clinical trial: cisplatin, the ansa macrolide antibiotic maytansine, and the nitrosourea chlorozotocin.

PATIENTS AND METHODS

Patients were stratified by Eastern Cooperative Oncology Group (ECOG) performance status (PS) (ambulatory, PS 0 or 1 versus nonambulatory, PS 2) as well as by disease extent (limited versus extensive).

To be eligible for entry in this study, each patient had to be refractory to standard chemotherapy and had to have measurable or evaluable disease, an ECOG PS of 0–2, a wbc count ≥ 4000/mm^3, a platelet count ≥ 100,000/mm^3, a BUN ≤ 25 mg/100 ml, a creatinine < 1.5 mg/100 ml, and a bilirubin ≤ 2.0 mg/100 ml. All patients gave informed written consent prior to participation in this study.

Patients randomized to cisplatin received 75 mg/m^2 iv every 21 days after adequate hydration and diuresis. Maytansine was given at 1.5 mg/m^2 iv every 21 days and chlorozotocin was given at 120 mg/m^2 iv every 42 days. Each drug was given until there was clinical evidence of progressive disease, after which patients were not eligible for treatment with the other study drugs.

Objective tumor response was classified according to the standard ECOG response criteria, and treatment complications were reported according to ECOG standard criteria by degree (none, mild,

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[1] Received June 26, 1981; revised Dec 10, 1981; accepted Jan 6, 1982.

[2] This study was conducted by the Eastern Cooperative Oncology Group (*P. P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in this study: American Oncologic Hospital, Fox Chase Cancer Center, Philadelphia, PA (*R. H. Creech*, CA-18281); Sidney Farber Cancer Institute, Boston, MA (*K. Stanley*, CA-23318); Albert Einstein College of Medicine, Bronx, NY (*S. E. Vogl*, CA-14958); The Johns Hopkins Oncology Center, Baltimore, MD (*D. S. Ettinger*, CA-16116); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (*P. D. Bonomi*, CA-10948); and University of Rochester Cancer Center, NY (*O. Salazar*, CA-11083). Other participating institutions include: Albany Medical College, NY (CA-06594); Baystate Medical Center, Springfield, MA (CA-20182); Case Western Reserve University, Cleveland, OH (CA-14548); Chicago Medical School, IL (CA-14144); Harbor General Hospital, Torrance, CA (CA-21091); Jefferson Medical College, Philadelphia, PA (CA-14215); University of Manitoba, Winnipeg, Canada; New York University, New York (CA-16395); Northwestern University Medical Center, Chicago, IL (CA-17145); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); University of Pretoria, South Africa (CA-21692); State University of New York-Stony Brook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); Wisconsin Clinical Cancer Center, Madison (CA-21076); and Yale School of Medicine, New Haven, CT (CA-15485).

[3] We thank Joann Keesey for excellent technical assistance.

**Reprint requests to:* Richard H. Creech, MD, American Oncologic Hospital, Fox Chase Cancer Center, Central and Shelmire Ave, Philadelphia, PA 19111.

moderate, severe, life-threatening, or lethal), type, and cause (1).

To avoid possible influences of imbalances caused by various prognostic factors, the analysis of the survival data was conducted using the proportional hazards model proposed by Cox (2–4).

RESULTS

Seventy-three patients were entered in this study between September 1978 and October 1979. Twenty-one of 25 patients admitted to the cisplatin arm, 19 of 25 patients admitted to the maytansine arm, and 18 of 23 patients admitted to the chlorozotocin arm were analyzed. Fifteen patients were considered unevaluable (seven had unacceptable laboratory values, the condition of five deteriorated prior to initiation of therapy, two had a PS > 2, and one refused treatment).

Of the 58 evaluable patients, all had previously received combination chemotherapy regimens: 21 had received one prior regimen, 25 had received two regimens, nine had received three regimens, two had received four regimens, and one had received seven regimens. The number of patients exposed to individual chemotherapeutic agents was as follows: 54, cyclophosphamide; 48, doxorubicin; 43, vincristine; 42, a nitrosourea; 34, methotrexate; 19, procarbazine; 15, etoposide; three, dacarbazine; two, hexamethylmelamine; one, 5-FU; and one, bleomycin. Sixty-five percent of these patients had previously had chemotherapy-induced responses.

Among the three treatment arms, there were no significant imbalances in patient characteristics including age, sex, extent of disease, initial PS, prior weight loss, and prior radiotherapy. Overall, 40% of the patients were ≥ 60 years old, 67% were male, 86% had disease extending beyond the hemithorax, 33% had a weight loss $\geq 5\%$ in the previous 6 months, 72% had prior radiotherapy, and 64% and 28% had a PS of 1 or 2, respectively.

There was only one partial response among the 58 evaluable patients. In this patient, one of 21 patients (5%) given cisplatin, a 1 × 1-cm skin metastasis underwent a complete regression by Day 25, while a rib metastasis remained stable for 53 days. Some degree of toxic reaction was observed in 100% of cisplatin-treated patients, in 79% of maytansine-treated patients, and in 72% of chlorozotocin-treated patients. Two patients receiving cisplatin had life-threatening thrombocytopenia (platelet count nadirs of 19,000 and 9500/mm^3, respectively). One patient receiving chlorozotocin had life-threatening gastrointestinal bleeding due to a platelet count of 20,000/mm^3. The severe toxic reactions associated with cisplatin included leukopenia (one patient), stomatitis (one), vomiting (two), and ototoxicity (one). There was no renal toxicity. Maytansine caused anemia requiring transfusions in one patient and severe neurologic toxic effects including myalgias, paresthesias, disorientation, headache, jaw pain, and weakness in four patients. One patient receiving chlorozotocin experienced severe stomatitis.

The 58 analyzed patients have died; they had an overall median survival of 9.7 weeks. The median survival times in weeks for the three regimens were 9.8 for cisplatin, 10.7 for maytansine, and 7.7 for chlorozotocin. Cisplatin-treated patients (P = 0.0[illegible]5) and maytansine-treated patients ($P = 0.02$) had longer survival times than chlorozotocin-treated patients. There was no significant difference in survival between the cisplatin- and maytansine-treated patients. Ambulatory patients (PS 0 or 1) had longer median survival times (10–12 weeks) than nonambulatory patients (PS 2; 5 weeks) ($P < 0.001$). The extent of disease and prior weight loss were not statistically significant prognostic indicators after adjusting for initial PS.

DISCUSSION

Although DeJager et al (5) reported four partial responses among 13 patients with small cell lung carcinoma treated with high-dose cisplatin (120 mg/m^2 every 3 weeks), cisplatin, maytansine, and chlorozotocin were not found to have significant antineoplastic activity in this phase II trial of patients with extensively previously treated small cell lung carcinoma refractory to chemotherapy. If an active agent is defined as one having a response rate of 20%, it can be calculated that we are 94% confident that the true response rate for this class of patients treated with cisplatin is $< 20\%$. For maytansine and chlorozotocin, the similar confidence levels are 99% and 98%, respectively. The most severe toxic effect of cisplatin and chlorozotocin was thrombocytopenia and for maytansine was neurologic. The unexpected thrombocytopenia noted with cisplatin most likely reflects chronic marrow suppression by previous extensive radiotherapy and chemotherapy in patients entered in this trial. The short survival times reflect the multiple previous treatments of these patients and the natural history of patients with progressive small cell lung carcinoma.

Because of the relatively short survival times of chemotherapy-refractory patients with small cell lung cancer, future phase II studies should be conducted only in ambulatory patients (ECOG PS 0 or 1) to allow adequate time for response evaluation.

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Combination Chemotherapy Used Prior to Radiation Therapy for Locally Advanced Squamous Cell Carcinoma of the Head and Neck[1]

Brief Reports

Ian Tannock,[2,*] Bernard Cummings,[2] Vicky Sorrenti,[2] and the ENT Group[3]

SUMMARY

Thirty-three patients with locally advanced squamous cell carcinoma of the head and neck were scheduled to receive two courses of chemotherapy prior to radical radiotherapy. Chemotherapy consisted of moderate-dose methotrexate with leucovorin rescue, bleomycin by infusion, and cisplatin. Loss of body weight and the duration of membrane formation at a specified region of the oral cavity during radiation therapy were used as indices of radiation toxicity: there was no excessive loss of body weight or mucosal reaction in patients who received combined treatment compared to patients in a nonrandomized control group who received radiotherapy alone. Twenty patients (60%) had a ≥ 50% decrease of measurable disease prior to starting irradiation, but only eight patients (24%) are alive and disease-free at a median followup of 16 months. Aggressive chemotherapy does not prevent delivery of subsequent full-dose radiotherapy for squamous cell carcinoma of the head and neck, but this study does not suggest that chemotherapy has a great beneficial effect on long-term survival.

[Cancer Treat Rep 66:1421–1424, 1982]

Patients with locally advanced squamous cell carcinoma (SCC) of the head and neck have a high rate of local failure after irradiation and/or surgery, and there is current interest in combining chemotherapy with these modalities. When chemotherapy has been given concurrently with irradiation, toxicity has usually been high, often leading to reduced dose or protracted delivery of radiation (1–3). Chemotherapy given prior to radiotherapy seems to be better tolerated (3–5), although the effect of combined modality treatment on mucosal toxicity has not been quantitated.

In the current study we have treated patients with locally advanced, node-positive SCC with two courses of aggressive chemotherapy followed by full-dose radiotherapy. Surgery was reserved for failure of initial therapy. End points of the study were toxicity from chemotherapy, weight loss and duration of mucosal reaction during radiation therapy in patients who received combined treatment compared with a nonrandomized group of patients receiving irradiation alone, response to chemotherapy at the time of starting irradiation, and disease-free survival.

STUDY DESIGN

Patients were eligible for this study if they had locally advanced, node-positive SCC of the head and neck (table 1) and were considered suitable for radiation treatment with curative intent. All patients were ambulatory (Eastern Cooperative Oncology Group performance status 0 or 1). They were assessed with physical examination including bidimensional measurements of lymph nodes, cbc, liver function tests, serum creatinine, chest X-ray, and tomograms of local disease. Patients with serum creatinine > 1.0 mg/100 ml had measurement of creatinine clearance and were excluded if this was < 60 ml/minute. Radiation dosage and fields were planned *before* institution of chemotherapy.

Eligible patients received overnight hydration (plus bicarbonate) before receiving the following iv chemotherapy: *Day 1*—methotrexate, 100 mg/m^2 every 3 hours for three doses and bleomycin, 20 units/m^2

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[1] Received Sept 10, 1981; revised Dec 22, 1981; accepted Jan 1982.

[2] The Princess Margaret Hospital and Ontario Cancer Institute, Toronto, Canada.

[3] F. Beale, R. Clark, A. Harwood, P. Fitzpatrick, T. Keane, D. Payne, and W. D. Rider, The Princess Margaret Hospital; M. Blackstein, The Mt. Sinai Hospital; D. Osoba, The Toronto Bayview Cancer Clinic; and D. Bryce and members of the Department of Otorhinolaryngology, University of Toronto, Ontario, Canada.

* *Reprint requests to:* Dr. Ian Tannock, The Princess Margaret Hospital and Ontario Cancer Institute, 500 Sherbourne St, Toronto, Canada, M4X 1K9.

by iv push; *Days 1–4*—bleomycin, 20 units/m²/day by continuous infusion; *Day 2*—leucovorin, 15 mg/m² every 6 hours for four doses, to start 24 hours after the first dose of methotrexate; and *Day 5*—cisplatin, 60 mg/m². Serum creatinine was measured daily; leucovorin administration was prolonged and cisplatin was omitted if serum creatinine increased significantly after methotrexate. Patients were seen weekly, and serum creatinine measurement and cbc were performed at this time. Patients were reassessed at 3 weeks: if there was no disease progression or severe toxic effect, they received one further identical course of chemotherapy beginning on Day 22 and commenced radiation treatment 2–3 weeks later. Patients with disease progression or toxic effects on Day 22 had no further chemotherapy and started radiation therapy as soon as practical. Patients were reassessed, with physical examination and X-rays defining disease, prior to the start of radiation therapy.

Radiation dose and fields depended on site of disease but were those usually prescribed for patients not receiving chemotherapy. Most patients were scheduled to receive radiation to sites of disease involvement as daily fractions ranging from 5000 rads/20 fractions/4 weeks to 6000 rads/30 fractions/6 weeks. Clinically normal lower cervical nodes received 3500 rads/15 fractions/3 weeks.

Patients were seen twice weekly during and immediately after radiation treatment and were weighed and questioned about oral intake and nausea and vomiting or other symptoms. The presence or absence of membrane formation on the buccal mucosa just anterior to the retromolar trigone was recorded (6). Patients were seen at least every 2 months following radiation therapy, and patients with residual or recurrent disease were assessed for surgery.

RESULTS

Toxic Effects of Chemotherapy

Twenty-four of the 33 patients completed two courses of chemotherapy; one patient received only one course because of disease progression, and eight had only one course because of toxicity. The most common reason for withholding the second course was a rise in serum creatinine: this occurred in seven patients, never exceeded 2 mg/100 ml, and reverted to normal in all patients. Hematologic toxicity was mild, and only three patients had polymorphonuclear leukocyte counts < 1000/mm³ or platelet counts < 50,000/mm³. There were no episodes of drug-related infection, although one patient required plate-

TABLE 1.—Distribution of patients by disease site, UICC stage (1974), and response to treatment*

Site	No. of patients	UICC stage				Response		Current Status		
		T_{1-2}, N_1	T_{1-2}, N_{2-3}	T_{3-4}, N_1	T_{3-4}, N_{2-3}	To chemotherapy	After irradiation	ADF	AWD	D
Nasopharynx	7	1	4	1	1	2 CR, 4 PR	5 CR, 2 PR	4	3	0
Oropharynx and oral cavity	12	1	1	2	8	7 PR	3 CR, 7 PR	2	6	4
Hypopharynx	7	2	2	1	2	3 PR	2 CR, 2 PR	1	1	5
Larynx	7	0	1	1	5	1 CR, 3 PR	2 CR, 3 PR	1	1	5

*UICC = International Union Against Cancer, ADF = alive and disease-free, AWD = alive with disease, and D = dead.

let transfusion. Most patients had nausea or vomiting after cisplatin. There was no mucositis, ototoxicity, or lung toxicity, although two patients had skin reactions to bleomycin, and one developed pericardial pain without effusion or other sequelae. During bleomycin infusion, many patients developed fever that was controlled with steroids. Myocardial infarction occurred in two patients during or shortly after drug treatment, and one of them had a cardiac arrest from which he was successfully resuscitated. Neither patient had a history of prior heart disease or hypertension.

Toxic Effects of Radiotherapy

Twenty-three patients completed radiation therapy as originally planned including two who had split-course therapy. Of the remaining ten patients, six had interruptions of 1–3 weeks because of toxic effects, one was treated with radical radiotherapy elsewhere, and one failed to return for radiation treatment until 3 months after chemotherapy. Only two patients did not receive radical-dose radiotherapy.

In 19 patients, the retromolar trigone region received the full radiation dosage. Membrane formation on the mucosa just anterior to the trigone occurred in 14 of these patients, and the median duration of membrane formation in all 19 patients was 24 days (range, 0–42). Duration of membrane reaction has been recorded (6) for a previously treated group of 13 patients with SCC of the oral cavity who received 5000 rads/20 fractions/4 weeks without chemotherapy, and the median duration of membrane formation in that series was 21 days (range, 0–24+). Our patients lost 10.0% ± 1.1% of their body weight (mean ± SEM) after combined treatment, of which 3.1% ± 0.6% occurred after chemotherapy and before the start of irradiation. The historical control group receiving radiation therapy alone lost a mean 6.2% of body weight (6).

Response to Chemotherapy

Twenty (60%) of 33 patients had objective response to chemotherapy when assessed just prior to starting treatment with radiation (table 1); three had complete response (CR) and 17 had partial response (PR). The criterion for PR was a 50% decrease in the product of two diameters of all measurable lesions (usually neck nodes), with radiologic or other evidence of regression in the primary site. CR required disappearance of all evident disease on physical and radiologic examination. Response rate was higher among the seven patients with nasopharyngeal cancer (two with CR, four with PR) than among patients with cancer at other sites.

Response to Combined Treatment

Disease status was recorded for 30 patients at completion of radiotherapy: using the same criteria for response as defined for chemotherapy, there were 12 patients with CR, 14 with PR, and four nonresponders. No patient had disease progression during radiotherapy, and 14 patients had objective regression of disease compared with their status after chemotherapy.

At a mean followup of 16 months (range, 9–20) after starting chemotherapy, only eight patients (24%) are alive and clinically free of disease (table 1). Seven of these patients originally responded to chemotherapy (one had CR and six had PR), and one has had surgery for residual disease after irradiation. Fourteen patients have died, 13 with their original disease and one with a second primary in the esophagus. Of the remaining 11 patients, nine are alive with disease, and two are lost to followup but were known to have disease when last seen.

DISCUSSION

This study was designed to examine whether aggressive chemotherapy given prior to radical radiotherapy for locally advanced SCC of the head and neck would influence radiation toxicity and to seek evidence for improvement in response and survival that might justify a larger study. Most of our patients had only minor and reversible toxic effects from chemotherapy and no evidence of increased toxicity from radiation therapy; this contrasts with reports of increased toxicity from concurrent administration of radiation and drugs (1–3).

Our initial 60% objective response rate to chemotherapy is consistent with that found in other series in which patients with SCC received chemotherapy prior to irradiation (3–5). Despite a high initial response rate to chemotherapy, the survival of patients in this trial remains poor, and only 24% of them remain disease-free at a median followup of 16 months. Clinically impressive objective responses to chemotherapy may represent only one decade of cell kill, and further evidence for a rather limited cell kill comes from our unpublished observations of a few patients with metastatic disease who had only short responses to the same chemotherapy when no subsequent irradiation was given. This rather small cell kill (on a logarithmic scale) added to the larger effect of irradiation may be insufficient to increase dramatically the probability of eliminating disease. Our results do not prove that chemotherapy has no benefit but suggest that large numbers of patients will be required in each disease and stage category if randomized trials are to have any chance of detecting a difference between patients who do and do not receive chemotherapy.

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Brief Reports

Phase II Study of Mitolactol and Semustine Combination Chemotherapy for Advanced Malignant Melanoma[1]

E. T. Creagan,* D. L. Ahmann, A. J. Schutt, and S. J. Green[2]

SUMMARY

Twenty-six patients with disseminated malignant melanoma received an oral regimen of mitolactol and semustine (methyl-CCNU). Sixteen patients had received prior systemic therapy, and 16 had visceral involvement. There were no objective regressions among ten patients receiving the drugs as initial systemic therapy. However, one of 16 patients having prior systemic treatment did have an objective response. Hematologic and gastrointestinal toxic effects were well-tolerated.

[Cancer Treat Rep 66:1425–1426, 1982]

Clinically meaningful chemotherapy for patients with disseminated malignant melanoma remains an elusive goal. Dacarbazine (DTIC), traditionally one of the more effective single agents, has achieved a response rate of only 14% (1). Multiagent regimens utilizing DTIC offer no consistent advantage over DTIC alone (2). The nitrosoureas have similarly displayed some antitumor activity against melanoma. In the absence of comparative clinical trials, the efficacy of carmustine (BCNU), lomustine (CCNU), or semustine (meCCNU) seems to be generally equivalent. MeCCNU is somewhat more attractive because of a 26% response rate among patients with primarily visceral-dominant disease (3) and a conveniently administered oral dose every 6 weeks.

Of considerable interest is a report which showed a 25% response rate from a daily oral dose of mitolactol (DBD), a halogenated hexitol, in patients with advanced malignant melanoma (4). The study is particularly noteworthy since responding patients had been previously treated with DTIC and a nitrosourea, a group notoriously resistant to subsequent chemotherapeutic efforts.

DBD plus meCCNU seemed to be an intriguing regimen for patients with advanced malignant melanoma. Each agent penetrates the central nervous system, a significant site for melanoma metastases, and clearly has activity in selected patients with disseminated disease. Experimental studies have shown striking therapeutic synergism in animal tumor models from the combination of dianhydrogalactitol (a related hexitol) and a nitrosourea (5). In addition, there is a reported precedent of enhanced therapeutic activity in melanoma by combining marginally effective agents with unusual mechanisms of antitumor activity (6). Halogenated hexitols are predominantly inhibitors of RNA metabolism (7), whereas nitrosoureas compromise purine nucleotide formation leading to inhibition of DNA synthesis.

We conducted a pilot study of the DBD and meCCNU combination among 11 patients with a spectrum of advanced solid tumors. Toxicity was primarily hematologic, with leukopenia and/or thrombocytopenia affecting six of 11 patients (table 1). Nausea and vomiting were transient and mild. A clinically acceptable dose was determined to be an oral regimen of 60 mg/m²/day of DBD and 135 mg/m²/day of meCCNU every 6 weeks.

MATERIALS AND METHODS

Twenty-six patients with biopsy-proven disseminated melanoma participated in the study. There were 11 males and 15 females, with a median age of 53.5 years. Fourteen patients had received prior chemotherapy, two had received prior hormone therapy, and ten had had no prior systemic therapy. Twenty-two patients had an Eastern Cooperative Oncology Group performance status of 0–1, and four had a status of 2–3; 16 patients had visceral-dominant disease, and ten had soft tissue or nodal involvement.

Response was defined as a ≥ 50% decrease in the product of the longest perpendicular diameters of

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[1] Received Sept 30, 1981; revised Dec 1, 1981; accepted Jan 6, 1982.

[2] Division of Medical Oncology (*E. T. Creagan, D. L. Ahmann,* and *A. J. Schutt*); and Cancer Center Statistics (*S. J. Green*), Mayo Clinic, Rochester, MN.

**Reprint requests to:* E. T. Creagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

TABLE 1.—Hematologic toxicity of DBD + meCCNU chemotherapy in a pilot study of 11 patients with advanced solid tumors

Dose (mg/m^2)		No. of patients	No. of courses	Nadir (cells × 10^3/mm^3)					
				Wbc count*			Platelet count†		
DBD	MeCCNU			Mean	Median	Range	Mean	Median	Range
40	90	5	6	7.7	6.5	2.3–15.7	312	337	42–822
40	135	3	4	8.0	9.2	3.4–13.3	264	251	23–468
60	135	3	3	8.4	7.3	3.6–18.0	269	114	65–692

*Normal wbc count: > 4.1 × 10^3/mm^3.

†Normal platelet count: > 130 × 10^3/mm^3.

the most clearly measurable indicator lesion without the development of new lesions or significant symptomatic deterioration. Disease progression was defined as a > 25% increase in the product of any lesion, the appearance of new lesions, or observation of progression by a local physician (for the one patient who did not return).

RESULTS AND DISCUSSION

There were no objective regressions among the ten patients who received DBD and meCCNU as initial treatment (table 2), whereas one of the 16 patients receiving the regimen as secondary systemic treatment had a regression. This patient, a 77-year-old woman, had been treated with PALA and later AMSA; she had a decrease in the size of soft tissue lesions for 6+ months. For all patients, the median survival and time to progression were 4.8 and 1.4 months, respectively.

Mild to moderate nausea, vomiting, or both occurred in 35% of patients. Thirty-eight percent of patients had leukopenia (< 4.1 × 10^3 wbcs/mm^3), thrombocytopenia (< 130 × 10^3 platelets/mm^3), or both. There were no clinically significant episodes of hemorrhage or infection, and there were no treatment-related deaths.

We were able to administer only approximately 50% of the generally accepted single-agent dose of each agent used in this regimen. In a prior study, which achieved apparent benefit from combination chemotherapy in advanced melanoma, each of the drugs having modest single-agent activity could be given at full dose (6). We recognize that only one third of our patients demonstrated any myelosuppression, and this could have had an impact on our findings. However, it is unclear if higher doses or alternative scheduling would substantially alter our results. Experimental studies show no apparent schedule dependency for a hexitol plus a nitrosourea (5), and the antitumor activity of a nitrosourea may be independent of myelosuppression (8).

In the dose and schedule used in this study, the combination of DBD and meCCNU displayed a small amount of activity against malignant melanoma. A 95% confidence interval for the regression probability is 0.001, 0.20. Surprisingly, the patient who responded was one who had failed two previous cytotoxic trials.

TABLE 2.—Results of treatment, by prior chemotherapy and site of metastasis, in 26 patients with disseminated melanoma*

Treatment	Visceral dominant	Nonvisceral
Primary	0/7	0/3
Secondary	0/9	1/7

*Values = No. of objective regressions/No. of evaluable patients.

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Brief Reports

Phase II Study of Methyl-GAG in the Treatment of Esophageal Carcinoma[1,2,3]

David Kelsen,* Robert Chapman, Manjit Bains, Robert Heelan, Mary Dukeman, and Robert Golbey[4,5]

SUMMARY

Twenty-four patients with epidermoid carcinoma of the esophagus have been treated, in a phase II trial, with methyl-GAG using a weekly schedule. Eighteen patients had received prior chemotherapy with cisplatin-containing combinations. Of 23 evaluable patients, four (17%) had partial remissions, each lasting 2 months. All patients had received prior chemotherapy. Toxic effects were manageable and included mild nausea and vomiting, mucositis, and fatigue. Using this schedule, methyl-GAG had modest activity in esophageal cancer, with acceptable toxicity. Its role in combination chemotherapy remains to be defined.

[Cancer Treat Rep 66:1427–1429, 1982]

Methyl-GAG is a hydrazone derivative which initially entered clinical trial in the mid-1960s. In these early studies, the drug was given as a rapid iv infusion at doses of 3.5–15 mg/kg on a daily treatment schedule. Although substantial antineoplastic activity was noted in patients with acute leukemias, lymphomas, and esophageal carcinoma (in a study by Falkson), toxicity was severe (1–6). Dose-limiting side effects included mucositis, skin ulcerations, diarrhea, and hypoglycemia. Myelosuppression was minimal. Because of its severe toxic effects, interest in the drug waned. Recently, Knight et al (7) restudied methyl-GAG using a weekly schedule. Antineoplastic activity was again seen in a variety of tumors, but toxic effects were markedly decreased. In view of its more acceptable toxicity, a phase II study in esophageal carcinoma, utilizing a weekly schedule, was performed. Preliminary results of this study have been previously reported (8).

MATERIALS AND METHODS

Twenty-four patients with advanced epidermoid carcinoma of the esophagus (the diagnosis having been confirmed by the Department of Pathology, Memorial Sloan-Kettering Cancer Center) were entered in this trial which began in November 1979 and ended in March 1981. All patients had measurable metastatic disease or recurrent tumor that was evaluable by barium esophagram. Methyl-GAG was supplied by the Drug Evaluation Branch of the National Cancer Institute (Bethesda, MD) in 1-g vials. It was reconstituted with 50 ml of normal saline immediately prior to use. The drug was then diluted in 250 ml of normal saline and infused over 1–1.5 hours. The initial dose was 400–500 mg/m^2 for poor-risk patients (defined as those with a Karnofsky performance status ≤ 60 or having had heavy prior chemotherapy) and 600–700 mg/m^2 for good-risk patients. Methyl-GAG was given weekly for 4 weeks. After a 1-week break, a second cycle was given, with a 100-mg/m^2 dose escalation if no significant toxicity had been noted during the first course. If severe toxicity was seen, methyl-GAG was held until the patient had recovered.

Prior to entrance in the study, all patients had a complete history and physical examination, chest roentgenogram, barium esophagram, electrocardiogram, urinalysis, creatinine clearance, and 12-channel biochemical screening profile. A cbc and platelet count and serum creatinine were repeated prior to each treatment. Because hypoglycemia had been noted in earlier studies, a serum glucose level was also measured before each dose.

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[1] Received Nov 16, 1981; accepted Jan 25, 1982.

[2] Supported in part by contract N01-CM-07337 from the Diision of Cancer Treatment, National Cancer Institute, National nstitutes of Health, Department of Health and Human Services.

[3] Presented in part at the 17th Annual Meeting of the American ociety of Clinical Oncology, Washington, DC, Apr 1981.

[4] Solid Tumor Service, Department of Medicine, Thoracic Surery Service, Department of Surgery, and the Department of Diagnostic Radiology, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY.

[5] We express our appreciation to Barbara Nagerl for assistance in preparing the manuscript.

**Reprint requests to:* David P. Kelsen, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

Response criteria, including radiologic examples of response as seen by barium esophagram, have been previously reported (9). An adequate trial was defined as at least three doses of methyl-GAG. Informed consent was obtained from all patients prior to entrance in the trial.

RESULTS

Of the 24 patients entered in the study, 23 were evaluable for response and toxicity. One patient was lost to followup after receiving only two doses of the agent. Patient characteristics are shown in table 1. Of note, most of these patients were heavily pretreated, usually with cisplatin and bleomycin or cisplatin, bleomycin, and vindesine combinations (9,10). As was the case with our earlier phase II trials, patients with poor performance status were initially accepted in the study.

Of the 23 evaluable patients, four (17%) had partial remissions, each lasting 2 months. All of these patients had received at least four courses of the cisplatin-containing regimens mentioned above. Three additional patients had minor regressions, which were not of substantial clinical benefit.

Toxicity

Nausea and vomiting were usually quite mild or absent. Alopecia was also uncommon. Mucositis was seen in two patients. Myelosuppression was not seen (median wbc count nadir, 7.6 × 10^3/mm^3 [range, 3–18.4]; median platelet count nadir, 595 × 10^3/mm^3 [range, 300–760]).

The dose-limiting toxicity was a syndrome of fatigue and weakness which was severe in four patients. This was usually seen after two to four doses and, if it occurred and the patient had stable disease or response, methyl-GAG was given every 2 weeks. The syndrome was usually ameliorated using this schedule.

Hypoglycemia was not seen.

DISCUSSION

Epidermoid carcinoma of the esophagus remains a difficult disease to treat. Few single-agent chemotherapeutic trials have been performed. Conventional drugs, such as bleomycin, methotrexate, doxorubicin, 5-FU, and lomustine (CCNU), have been studied in small groups of patients and have had minimal or no activity (11–15). Initial studies from Memorial Hospital starting in 1976, involving investigational drugs, demonstrated modest activity for the two-drug combination of high-dose cisplatin and bleomycin infusion, with 19% of patients having complete or partial regressions (9). In patients with metastatic disease, the median duration of response was 6 months. Since this study, two trials of cisplatin alone involving a total of 36 patients have been reported; the overall response rate was 18%, with brief remission durations (16,17).

TABLE 1.—Patient characteristics

Characteristic	No. of patien
Total entered	24
Evaluable	23
Median age in yrs (range): 59 (48–80)	
Median performance status (range): 70 (30–80)	
Sex (male:female)	19:5
Prior therapy	
None	1
Chemotherapy	9
Radiation therapy	5
Chemotherapy and radiation therapy	9

A phase II study with the new vinca alkaloid, vindesine, followed our trial with cisplatin and bleomycin. As was the case with the current study involving methyl-GAG, most of these patients were heavily pretreated, usually with the cisplatin and bleomycin combination. An 18% complete and partial response rate was noted, with a 5-month median duration of response (18). Because they have differing mechanisms of action and toxic effects, and appear, at least in some patients, to lack cross-resistance, the three agents (cisplatin, vindesine, and bleomycin) were then combined. This study has now accrued 62 evaluable patients, 19 with metastatic disease and 43 with local-regional disease (10). The complete and partial response rate is 55%; in patients with metastatic disease, the median duration of remission will be approximately 7.5–8 months. Although the more than doubling of the response rate and increased response duration are quite encouraging, it still means that 40%–50% of patients are not responding to our best chemotherapy combination. Thus, the activity of methyl-GAG, although modest, is of interest, especially in view the prior chemotherapy these patients had received. Whether or not methyl-GAG can be incorporated into a cisplatin-vindesine-bleomycin type combination will depend on the results of a phase trial, currently in progress.

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Brief Reports

Phase II Study of Chlorozotocin in Malignant Melanoma: A Southeastern Cancer Study Group Report[1,2]

A. L. Van Amburg, III,[3] Cary A. Presant,[4,*] and Debora Burns[5]

SUMMARY

Seventy-nine patients with metastatic melanoma received chlorozotocin (150 mg/m^2) every 6 weeks. Two complete and five partial responses were observed: 18% in patients who had not previously received chemotherapy and 6% in previously drug-resistant patients. Dose-limiting toxic effects were vomiting and myelosuppression.

[Cancer Treat Rep 66:1431–1433, 1982]

Chlorozotocin is a nitrosourea that was shown in phase I studies (1–3) to be only minimally myelosuppressive at doses of 120–175 mg/m^2. It had possible activity against malignant melanoma (1,2). In a recently reported phase II study, a response rate of 14% (five responses among 35 patients) was observed in patients with metastatic malignant melanoma (4). To extend that observation, we report here the experience of the Southeastern Cancer Study Group using chlorozotocin in malignant melanoma.

MATERIALS AND METHODS

Seventy-nine patients with incurable malignant melanoma were entered in the study between January 1978 and February 1981. Nine patients were inevaluable because of insufficient data or protocol violation. Of the remaining 70 patients, 47 were previously treated with chemotherapy (excluding nitrosoureas) and 23 were previously untreated. Voluntary informed consent had been given prior to treatment.

Chlorozotocin (150 mg/m^2) was given iv every 6 weeks. Complete response (CR) was defined as the complete disappearance of all manifestations of tumor; partial response (PR) was defined as a 50%–100% decrease in the sum of the products of perpendicular diameters of all measured tumors, without the appearance of new tumors; stable disease was defined as less than a PR, with no evidence of disease progression; and disease progression was defined as the appearance of new manifestations of tumor or a > 25% increase in measured tumor area.

Myelotoxicity was defined as follows: grade 1 included a wbc count nadir of 3000–4000/mm^3 or a platelet count nadir of 100,000–130,000/mm^3; grade 2 included nadirs of 2000–3000 or 50,000–100,000/mm^3, respectively; grade 3 included nadirs of 1000–2000 or 25,000–50,000/mm^3, respectively; and grade 4 included nadirs < 1000 or < 25,000/mm^3, respectively. Dose reductions of 50% were instituted for grade 3 or 4 myelotoxicity. No course was given until the wbc count was > 4000/mm^3 and the platelet count was > 100,000/mm^3.

RESULTS

The results of the study are summarized in table 1. In the group of 23 previously untreated patients, two patients (9%) had CR lasting 6 and 18 months, and two (9%) had PR lasting 1.5 and 3+ months. Four patients had stable disease for 1+, 1.5, 3+, and 4 months. The total response rate was 18% in previously untreated patients. Nine of the 23 patients had visceral metastases, and four (44%) of the nine had CR or PR.

There were only three patients (6%) with PR and four (9%) with stable disease among 47 patients who had previously received chemotherapy. The durations of PR were 3+, 4+, and 6 months, and the durations of stable disease were 1.0, 1.5, 1.5, and

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[1] Received Dec 21, 1981; accepted Jan 26, 1982.

[2] Supported in part by Public Health Service grants CA-19657, CA-24456, CA-17027, CA-02055, CA-07961, CA-12223, CA-03177, CA-05641, CA-13237, CA-23909, CA-03376, CA-30247, CA-28138, and CA-28143 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Jewish Hospital of St. Louis, MO.

[4] Department of Medical Oncology, City of Hope National Medical Center, Duarte, and Wilshire Oncology Medical Group, Los Angeles, CA.

[5] University of Alabama, Birmingham.

**Reprint requests to:* Cary A. Presant, MD, Department of Medical Oncology, City of Hope National Medical Center, 1500 E Duarte Rd, Duarte, CA 91010.

TABLE 1.—Response to chlorozotocin

	Total No. of patients	No. of patients (%)				
		CR	PR	Stable disease	Disease progression	Death
Previously untreated	23	2 (9)	2 (9)	4 (18)	13 (56)	2 (9)
Previously treated	47	0	3 (6)	4 (9)	32 (68)	8 (17)

2+ months. Two (18%) of the 11 patients with visceral metastases had PR, and one (3%) of the 36 patients with cutaneous and/or nodal melanoma had PR.

A tabulation of the responding patients is given in table 2. All but one of the seven patients who had CR or PR had visceral metastases in lung or liver. Both patients who had CR had liver metastases. No patient with a low performance status (< 60%) had a PR, but three of the seven responders (including both with CR) had a pretreatment performance status of only 70%.

There were 118 courses of chlorozotocin evaluated for toxicity. Thirteen episodes of grade 2 leukopenia and five episodes of grade 3 leukopenia occurred. One patient developed sepsis during leukopenia. There were 13 episodes of grade 2 and five episodes of grade 3 thrombocytopenia. The lowest platelet count nadir was 10,000/mm^3.

Eight patients received four or more courses of chlorozotocin that were evaluated for toxicity. Three of these patients developed grade 2 thrombocytopenia after the second course and two developed grade 2 leukopenia; two of the episodes occurred in the previously untreated patients. There were six patients with decreases in hemoglobin > 1 g/dl. Two previously treated patients had severe decreases in hemoglobin (nadirs, 6.4–6.7 g/dl) during the first course of therapy.

One patient had drug-related hepatitis and one had drug-related dyspnea, both associated with chlorozotocin administration, which required discontinuation of the drug. Nausea and vomiting were extremely common, although they usually lasted only several hours and were partially responsive to antiemetics. Nine patients had vomiting unresponsive to antiemetics, and one became dehydrated due to intractable vomiting.

DISCUSSION

Chlorozotocin has been shown to be an active drug in disseminated malignant melanoma, with two CRs and a total response rate of 18% in previously untreated patients. This is similar to the 15% response rate reported by Hoth et al (4) in 20 previously untreated patients using chlorozotocin at a dose of 120 mg/m^2. It is significant that CRs were seen in both studies.

These results contrast somewhat with those of Houghton et al (5). In their study of 19 patients, only one response (less than a PR) was observed in 15 patients with prior chemotherapy, and there were no responses in four previously untreated patients (despite use of chlorozotocin at doses of 175–200 mg/m^2). The response rate in previously drug-resistant patients is not different from that which we ob-

TABLE 2.—Characteristics of patients with CR or PR

Accession No.	Prior chemotherapy	Pretreatment performance status	Sites of tumor metastases	Response	Duration of response (mos)
11002	No	70%	Liver, nodes	CR	18
11022	No	70%	Liver, lung, nodes, skin	CR	6
29005	No	80%	Lung, small intestine	PR	3+
29001	No	90%	Liver, nodes	PR	1.5
23001	Yes	70%	Lung, bone, skin	PR	6
28005	Yes	100%	Lung	PR	4+
22002	Yes	90%	Nodes	PR	3+

served (6%). Since only four patients had not received prior drugs, the failure of Houghton et al to observe any responses is not significant and is consistent with our response rate of 18%.

More myelotoxicity was seen in this study, which used a 25% higher dose of chlorozotocin, than was seen in the previous report by Hoth et al (4). However, we observed less myelosuppression than did Houghton et al (5), who used doses of 175–200 mg/m^2. In this series, there was evidence of cumulative myelosuppression in some patients who received four or more courses.

We conclude that although some activity of chlorozotocin has been observed (which is similar to that of other nitrosoureas), the only meaningful role of chlorozotocin in management of malignant melanoma will likely be in combination with other more myelosuppressive chemotherapeutic agents. In such trials of combinations, chlorozotocin cannot be regarded as a nonmyelosuppressive drug. Cautious phase I trials to define the maximally tolerated dose of the combinations containing chlorozotocin will be required to avoid severe or cumulative myelosuppression.

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Changes in Intestinal Permeability to Lactulose Induced by Cytotoxic Chemotherapy[1]

Brief Reports

Gianpaolo Parrilli, R. Vincenzo Iaffaioli, Gaetano Capuano, Gabriele Budillon, and A. Raffaele Bianco[2,*]

SUMMARY

Intestinal function during cytotoxic therapy was studied in nine patients with nonintestinal lymphoma. An early, marked, and rapidly reversible increase in passive permeability, as disclosed by the lactulose test, was observed without concomitant changes in active absorption or in intestinal histology. The possibility of a toxic effect by cytotoxic drugs on intestinal intracellular junctions is discussed.

[Cancer Treat Rep 66:1435–1436, 1982]

The high cellular proliferation rate of the intestinal epithelium makes it particularly vulnerable to the effects of cytotoxic drugs and ionizing radiation (1,2). Structural and functional alterations in the mucosa of the small intestine have been described in animals and in man during treatment with antimetabolites such as aminopterin (3,4), methotrexate (5), and 5-FU (6) and with colchicine (7,8) and cyclophosphamide (9).

We studied intestinal permeability as previously described (10), using Menzies' lactulose test (11,12), in nine patients with nonintestinal malignant lymphoma who were receiving cytotoxic therapy. Five patients with Hodgkin's disease were treated with the MOPP regimen (13), and four patients with non-Hodgkin's lymphoma were treated with the CVP regimen (14).

The lactulose test measures by quantitative paper chromatography (15) the minute fraction of lactulose excreted in urine 5 hours after administration of this sugar by hyperosmolar load (7 g of lactulose plus 40 g of sucrose in 100 ml of water). The test was performed before and then 2, 10, 16, and 30 (MOPP patients only) days after the beginning of cytotoxic therapy. In four patients a xylose test and an intestinal biopsy were done before and 10 days after the beginning of treatment.

The results are summarized in table 1. Cytotoxic therapy caused immediate repercussions on intestinal permeability to lactulose, which was more than doubled in all subjects as early as the second day of therapy. Subsequently, the test showed a gradual return to basal conditions. However, the xylose test and intestinal histology were unaltered.

The permeabilization effect observed did not seem to have been associated with a hyporegenerative mechanism. Epithelial hyporegenerative damage would have resulted in a decrease in active transport of xylose and an increase in passive permeability to lactulose, such as that described in villous atrophy of celiac disease (16), with maximum evidence after 7–10 days of cytotoxic therapy. In fact, cytotoxic drugs can only block cellular mitosis in the regenerating zone of the epithelium at the crypt level, and cell migration from the crypt floor to the apex of the intestinal villous is known to take from 5 to 7 days (17,18). During this period the pre-existing differentiated epithelium continues to maintain normal intestinal function.

The early appearance and prompt resolution of the permeability increase to lactulose is better explained by a transient metabolic disturbance. It has been suggested that the intestinal transfer of lactulose occurs via channels associated with junctional complexes between adjacent enterocytes (19). The absorption of lactulose can be potentiated by ingestion of a hypertonic solution (11). The hypertonicity is postulated to increase permeability by making intracellular tight junctions more leaky (11,16). The cytotoxic combination therapy employed may exert a further temporary weakening effect on the same junctions. A study of single cytotoxic drugs and different combinations of sugars as intestinal permeability markers is in progress and should clarify the mechanisms involved.

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[1] Received Aug 20, 1981; revised Jan 21, 1982; accepted Jan 29, 1982.

[2] Institute of Clinical Medicine (*G. Parrilli, G. Capuano,* and *G. Budillon*) and Division of Medical Oncology (*R. V. Iaffaioli* and *A. R. Bianco*), University of Naples Medical School II, Italy.

[*] *Reprint requests to*: A. Raffaele Bianco, MD, Division of Medical Oncology, University of Naples Medical School II, Via S Pansini 5, 80131, Naples, Italy.

Table 1.—Lactulose excretion during cytotoxic therapy

Patient No.	Type of lymphoma	Therapy	Urinary lactulose basal level (mg/5 hrs)	% permeability increase (Day)				Xylose test	Intestinal biopsy
				2	10	16	30		
1	Hodgkin's	MOPP	68	142	47	9	3	Normal	No change
2	Hodgkin's	MOPP	55	94	49	27	12		
3	Hodgkin's	MOPP	70	78	55	0	0		
4	Hodgkin's	MOPP	54	107	74	0	14	Normal	No change
5	Hodgkin's	MOPP	72	106	31	0	0		
6	Non-Hodgkin's	CVP	84	116	0	0		Normal	No change
7	Non-Hodgkin's	CVP	48	110	25	0			
8	Non-Hodgkin's	CVP	70	85	17	0			
9	Non-Hodgkin's	CVP	80	100	0	0		Normal	No change

Our data suggest that as an indicator of altered intestinal function during cytotoxic therapy, the lactulose test could be more sensitive than either intestinal biopsy or tests that measure active intestinal transport.

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Clinical
Trials
Summaries

Phase II Study of Tamoxifen in Patients With Metastatic Malignant Melanoma[1]

Ragnar Telhaug,* Olbjørn Klepp, and Ole Børmer[2]

Several reports have presented evidence of estrogen receptor activity in tumor tissue from 12% to 46% of patients with malignant melanoma (1). Nesbit et al (2) reported in 1979 that four of 26 (15%) patients with advanced malignant melanoma treated with tamoxifen (TAM) had an objective tumor response. Prompted by this report, in December 1979 we initiated a phase II study of TAM in patients with disseminated malignant melanoma, and we report our results here in order to contribute to the overall estimation of the collective response rate.

Twenty-one patients with measurable, disseminated malignant melanoma were included in the study (table 1). Tumor tissue samples for the determination of cytosol hormone receptor concentrations were obtained from seven of the patients before the start of TAM therapy. The patients received TAM at a daily dose of 40 mg/day orally in two divided doses until clear evidence of tumor progression. Prior to initiating TAM therapy and at least every 4–6 weeks during the study period, patients had a complete clinical evaluation, chest roentgenogram, and routine hematologic and blood chemistry tests.

One of the patients achieved an objective improvement. He had lung metastases that decreased > 50% in size after 2 months of TAM treatment. Disease progression occurred 5 months after the start of TAM therapy. Two patients had no change in their tumor lesions for 4 and 5 months, respectively. In both cases the rate of disease progression was comparatively slow both before and after TAM therapy. All of the other 18 evaluable cases showed disease progression within 2–3 months after the start of TAM therapy. None of the seven tumor tissues examined showed estradiol binding activity. No significant side effects of TAM therapy were experienced by any of the 21 patients.

The rate of complete and partial response to TAM in advanced malignant melanoma has not been impressive (3,4). In our study we found one responder among 21 evaluable patients. But even a 5%–10% response rate could be of some clinical interest for the therapy of malignant melanoma, because the side effects of such therapy are negligible compared to those of cytotoxic drugs. So far, the possible therapeutic effect of TAM in malignant melanoma remains uncertain at best.

TABLE 1.—Summary of characteristics of 21 patients treated with TAM

Characteristic	No. of patients
No. of evaluable patients	21
Median age in yrs (range): 55 (29–80)	
No. of males/females	12/9
Prior chemotherapy	8
Performance status	
80–100	10
50–70	9
< 50	2
Dominant metastatic site	
Cutaneous, subcutaneous, and/or lymph nodes only	14
Lung	5
Liver	1
Bone	1
Results	
Partial response	1 (5 mos)
Stable disease	2 (4 and 5 mos)
Disease progression	18 (within 2–3 mos)

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[1] Received Aug 10, 1981; revised Dec 9, 1981; accepted Jan 6, 1982.

[2] The Norwegian Radium Hospital, Oslo, Norway.

**Reprint requests to:* Dr. Ragnar Telhaug, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway.

Clinical Trials Summaries

Combination Chemotherapy With Bleomycin and Hydroxyurea in the Treatment of Advanced Head and Neck Cancer[1]

Mario A. Eisenberger,* John Denefrio, Michael Silverman, and Howard E. Lessner[2]

Bleomycin has been shown to cause kinetic perturbations (1,2) and to produce partial cell synchronization in an in vivo melanoma study (1). A 24-hour bleomycin infusion (3) followed by hydroxyurea has been reported to produce a significant (59%) response rate in patients with head and neck cancer. We report here a study of this combination adjusted for use in an outpatient environment.

METHODS

Twenty-four patients with histologically proven squamous cell carcinoma of the head and neck were treated; all patients had measurable disease. Responses were defined as follows: *complete response*—the complete disappearance of all measurable lesions, with pathologic confirmation; *partial response*—a ≥ 50% decrease in the sum of the products of the largest perpendicular diameters of measurable lesions without the appearance of new lesions; and *stable disease*—a < 50% decrease or a < 25% increase in measurable parameters for at least 8 weeks. Time to progression and survival time were measured from Day 1 of therapy.

Bleomycin was given at a dose of 15 units/m^2 iv on Day 1, followed by hydroxyurea on Day 8 through Day 15 at a daily oral dose of 1000 mg/m^2 in divided doses, repeated every 2 weeks. After a total dose of bleomycin (300 units) was reached, hydroxyurea alone was continued at the same dose daily or as tolerated until progression of disease.

RESULTS

Of the 24 patients entering the study, 19 were evaluable. Four patients were lost to followup within 4 weeks of therapy, and one patient with evidence of response was inadvertently started on palliative irradiation to the indicator lesion during chemotherapy. Table 1 summarizes patient characteristics, response rates, time to disease progression, and survival. Initial Karnofsky performance status was > 70% for patients who had partial response or stable disease and 50%–60% for nonresponders.

Toxic effects were mild to moderate, with two

TABLE 1.—Patient characteristics and response*

Characteristic	
Sex	
Male	18
Female	1
Primary sites	
Unknown	3
Larynx	5
Oropharynx	3
Floor of the mouth	1
Posterior tongue	1
Anterior pilar	1
Tonsil	1
Nasopharynx	1
Oral cavity, not specified	3
Extension of disease (response)	
Loco-regional	16 (9 PR, 3 SD)
Distant metastatic (below the clavicle)	3 (2 SD)
Response†	
PR	9 (48%)
SD	5 (31%)
Disease progression	5 (31%)
Median time to progression (days)	
PR	130
SD	73
Disease progression	42
Median survival (days)	
PR	250
SD	142
Disease progression	112

*Unless specified otherwise, values = No. of patients. PR = partial response; SD = stable disease.

†Response rates on the basis of all 24 patients entered in the study are: PR, 38% (9/24); SD, 20% (5/24); and disease progression, 20% (5/24).

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[1] Received Aug 31, 1981; revised Dec 14, 1981; accepted Jan 5, 1982.

[2] Department of Oncology, Comprehensive Cancer Center for the State of Florida, Miami.

**Reprint requests to:* Mario A. Eisenberger, MD, Cancer Therapy Evaluation Program, National Cancer Institute, Landow Bldg, Rm 4A16, 7910 Woodmont Ave, Bethesda, MD 20814.

patients developing wbc count nadirs of < 1000/mm^3 and 12 developing nadirs between 1000 and 3000/mm^3. Platelet count ranged from 50,000 to 100,000/mm^3 in three patients. Radiologic evidence consistent with pulmonary fibrosis was noted in one patient (total dose of bleomycin, 300 units). Fever and chills were seen in 16 of 19 patients after bleomycin, and one patient had hydroxyurea-induced nausea and vomiting.

DISCUSSION

Bleomycin used alone on a weekly or twice-weekly schedule produces responses similar to those seen in our series (4), whereas hydroxyurea alone seems to have only marginal activity (4). Although our results do not allow us to make any conclusions about the synergism of this combination, the less intensive schedule in this pilot study produced responses in a significant number of patients. It appears at least as effective as more intensive and/or toxic regimens in the palliation of patients with advanced squamous cell carcinomas of the head and neck.

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High-Dose Medroxyprogesterone Acetate for the Treatment of Advanced Ovarian Carcinoma[1]

Clinical Trials Summaries

Claes Tropé,* John-Erik Johnsson, Kristján Sigurdsson, and Ernst Simonsen[2]

High-dose medroxyprogesterone acetate (MPA) is of significant value in palliative treatment of hormone-dependent neoplasms such as breast (1) and endometrial (2) carcinoma. Progestogens of various kinds have been reported to have activity in patients with ovarian adenocarcinoma, but the results have been conflicting, and the response rates have varied from 5% to 65% (3,4). The aim of this study was to further elucidate the effect of high-dose MPA in ovarian cancer resistant to cytostatic drugs.

Twenty-eight patients with recurrent, advanced (International Federation of Gynecology and Obstetrics stages III and IV), chemotherapy-resistant ovarian carcinoma, with measurable or evaluable lesions, were included in the study. Patient characteristics, response, survival, and toxic effects are shown in table 1.

MPA, a synthetic steroid derived from progesterone (Farmitalia Carlo Erba), was given im in a daily dose of 500 mg for 3 weeks, followed by 500 mg once a week for at least 2 months. Patients with an objective response or stable disease continued treatment with 500 mg every week until disease progression. The results of treatment were defined according to the World Health Organization criteria.

Three patients were not eligible due to early death from tumor. Twenty-five patients with a median age of 61 (range, 43–81) were evaluable; seven patients had stage III and 18 had stage IV disease.

Of the 25 evaluable patients, one achieved partial response (duration, 3+; survival, 4+ months); nine had stable disease (median duration, 5.5+ months; median survival, 6.5+ months); and 15 had progressive disease (median survival, 3 months). The objective response occurred in a 73-year-old woman with undifferentiated carcinoma, stage IV disease. This patient had a > 50% reduction of central pelvic mass and inguinal nodes. Nine (36%) of the 25 patients increased their Karnofsky index by a mean of 20 points.

During drug therapy, there were no changes in blood counts or serum levels of creatinine, bilirubin, alkaline phosphatase, and fasting blood sugar. One patient gained weight due to fluid retention and she also developed high blood pressure. More severe complications such as deep vein thrombosis were seen in two patients, but both had large pelvic tumors and had perhaps developed thrombosis.

The overall response rate was 4%, which is about the same result achieved by Malkasian et al (3) but far from the high response rate of 60% reported by Ward (4). Since most of the epithelial ovarian carcinoma cell types were included in this study, it is reasonable to conclude that objective responses may be obtained only in a minority of patients with ovarian carcinoma resistant to cytostatic drugs. However, we have used MPA as a third-line therapy and when chemotherapy is used as a second- or third-line therapy, the overall response rate is also very low (5).

The present investigation shows the MPA in patients with ovarian cancer previously treated with chemotherapy is not an effective treatment.

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[1] Received Sept 24, 1981; revised Dec 29, 1981; accepted Jan 11, 1982.

[2] Gynecologic Section, Department of Oncology, University Hospital, Lund, Sweden.

**Reprint requests to:* Dr. Claes Tropé, Gynecologic Section, Department of Oncology, University Hospital, S-221 85 Lund, Sweden.

TABLE 1.—Pretreatment features and response to MPA therapy*

Patient No.	Age	Histology	Surgery	Previous therapy		Clinical stage	Response	Duration (mos)		Pretreatment-posttreatment Karnofsky index	Side effects
				Irradiation	Chemotherapy			Remission	Survival		
1	73	AC	SOEB	+	Melf	IV	PR	3+	4+	80–100	None
2	45	SMD	HIT + SOEB	+	Melf + Adr	IV	SD	6+	7+	80–100	H + WG
3	63	SMD	Expl lap	+	Melf + Adr	IV	SD	6+	7+	80–100	None
4	67	SMD	SOEB	+	Melf	III	SD	6+	7+	80–80	None
5	69	AC	Expl lap	+	Melf	III	SD	7+	8+	100–100	T
6	68	SMD	Expl lap	+	Melf	III	SD	5+	6+	80–80	None
7	58	SPD	Expl lap	+	Melf	III	SD	5+	6+	80–100	None
8	74	SHD	SOEB	–	Melf + Adr	III	SD	4+	5+	80–100	None
9	69	SHD	HIT + SOEB	–	Melf + Adr	IV	SD	3+	4+	80–100	None
10	76	AC	Expl lap	+	Melf + Adr	IV	SD	3+	4+	80–100	None
11	43	EHD	SOEB	–	Melf + Adr + Cis	IV	PD		6	60–60	None
12	61	AC	FB	–	VM-26	IV	PD		6+	80–100	None
13	63	AC	Expl lap	–	Melf + Adr	IV	PD		6+	80–100	None
14	73	AC	FB	–	VM-26	IV	PD		5	80–80	T
15	61	AC	HIT + SOEB	+	Melf	IV	PD		3	80–80	None
16	61	AC	Expl lap	–	Melf	III	PD		3	50–50	None
17	70	AC	Expl lap	–	Melf + Adr + Cis	IV	PD		3	80–80	None
18	59	SPD	SOEB	–	Melf + Adr	IV	PD		3	80–80	None
19	81	AC	SOEB	+	Melf	III	PD		4+	80–80	None
20	55	SHD	SOEB	–	Melf + Adr	IV	PD		3	80–80	None
21	50	SPD	HIT + SOEB	+	Melf + Adr + Cis	IV	PD		3	60–60	None
22	50	SPD	HIT + SOEB	–	Melf + 4-epi	IV	PD		3	60–60	None
23	50	SPD	SOEB	+	Melf	IV	PD		3	60–60	None
24	49	EHD	HIT + SOEB	–	Melf + Adr	IV	PD		3	60–60	None
25	43	SPD	SOEB	–	Melf + Adr	IV	PD		3	80–80	None

*AC = anaplastic carcinoma, SMD = serous moderately differentiated, SPD = serous poorly differentiated, SHD = serous highly differentiated, EHD = endometrioid highly differentiated, SOEB = salpingo-oophorectomy, HIT + SOEB = total hysterectomy + salpingo-oophorectomy, Expl lap = explorative laparotomy, FB = fine needle biopsy, Melf = melphalan, Adr = doxorubicin, Cis = cisplatin, 4-epi = 4′-epi-doxorubicin, VM-26 = teniposide, PR = partial response, SD = stable disease, PD = progressive disease, H = hypertension, WG = weight gain, and T = deep thrombosis.

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Trial of Tamoxifen at a Dose of 40 mg Daily After Disease Progression During Tamoxifen Therapy at a Dose of 20 mg Daily[1]

Clinical
Trials
Summaries

J. F. Stewart,* M. J. Minton, and R. D. Rubens[2,3]

Tamoxifen, an antiestrogen, is an effective treatment in a number of postmenopausal, and probably premenopausal, women with advanced breast cancer (1). Although it has been suggested that higher response rates are seen in patients treated with 40 mg daily rather than 20 mg daily (2) and although there has been a case report of further disease remission with 20 mg twice daily after disease progression during therapy with 10 mg twice daily (3), the optimum dosage has not been established.

We have conducted a prospective nonrandomized trial to assess the response of advanced breast cancer to 20 mg of tamoxifen twice daily after disease progression during therapy with 10 mg twice daily.

The patients studied had histologically confirmed, locally advanced inoperable or metastatic breast cancer that was progressing during treatment with 10 mg of tamoxifen twice daily with or without 5 mg of prednisolone twice daily. After physical examination (with measurement of all palpable lesions and photography of visible lesions), chest radiograph, cbc, biochemical screen (including liver function tests), and isotopic bone scan (with radiographs of areas of increased uptake), the dose of tamoxifen was increased to 20 mg twice daily. Response was assessed by International Union Against Cancer criteria (4). Estrogen receptor (ER) analysis had previously been determined by the method of King et al (5). Patients with rapidly progressing visceral disease after the lower dose of tamoxifen were treated directly with chemotherapy and excluded from this trial.

Thirty-six consecutive patients were entered in this study; 30 were evaluable. Six patients were inevaluable for the following reasons: no assessable lesions (one patient), failure to take treatment because of side effects (three), loss to followup (one), and early death (one). Characteristics of the 30 evaluable patients are shown in table 1.

No objective responses to the higher dose of tamoxifen were observed. Seven patients had stable disease for 2–15 months, two who had responded to previous endocrine therapy (tamoxifen, prednisolone) and five who had had no prior response. These patients comprised two with ER+ (≥ 5 fmols/mg of cytosol protein) tumors, one with an ER– (< 5 fmols/mg of cytosol protein) tumor, and four with unknown tumor ER status. The other 23 patients all had progressive disease. Median time to treatment failure was 2 months (range, 1–15), and median survival from study entry was 11 months (range, 2–17).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	30
Mean age at entry in study, in yrs: 64.9	
Stage at presentation	
I or II	15
III or IV	15
Median disease-free interval, in mos (range): 20 (0–204)	
ER status*	
+	16
–	2
Not known	12
Previous systemic therapy for advanced disease	
Tamoxifen	25 (9)†
Tamoxifen + prednisolone	5 (5)
Estrogens	2 (1)
Androgens	2 (1)
Prednisolone	4 (2)
Progestogens	1 (1)
Chemotherapy	7 (3)
Sites of disease	
Soft tissue	26
Bone	5
Viscera	7

*+ = ≥ 5 fmols/mg of cytosol protein; – = < 5 fmols/mg of cytosol protein.

†Values in parentheses = No. of responses.

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[1] Received Dec 18, 1981; accepted Jan 25, 1982.

[2] Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, London, England.

[3] We thank R. J. B. King for performing the estrogen receptor analysis.

**Reprint requests to:* J. F. Stewart, MB, Imperial Cancer Research Fund, Breast Cancer Unit, Guy's Hospital, London SE1 9RT, England.

These results indicate that escalation of tamoxifen dosage to 20 mg twice daily after disease progression during therapy with 10 mg twice daily does not lead to further response and that alternative therapy, either endocrine or cytotoxic, should be given.

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Clinical Trials Summaries

Phase II Trial of Tamoxifen in Malignant Melanoma[1,2]

Cynthia G. Leichman, Michael K. Samson,* and Laurence H. Baker[3]

In 1976, Fisher et al (1) initiated a study testing for the presence of hormone receptors in melanoma biopsy specimens. The underlying premise that melanoma might be a hormonally sensitive tumor was based on observations that the natural history of the disease differed in men and women. Subsequent to the demonstration of cytoplasmic estrogen receptors in melanoma, several studies were initiated using hormonal manipulation in the treatment of disseminated malignant melanoma. Early studies suggested that regression of disease could be achieved with estrogen compounds (2).

Following the results of treatment of hormonally sensitive breast cancer with antiestrogens, similar therapy was attempted in disseminated melanoma. Several studies have produced mixed results ranging from no response to stabilization of disease to brief partial responses in small numbers of patients to a sporadic complete response (3–5). Increasing the doses of tamoxifen to obtain higher initial blood levels was no more successful in producing response. These studies revealed that the presence or absence of hormone receptors had no relation to clinical response or median survival (3). Performance status at the initiation of therapy was the most significant predictor of survival.

Between 1978 and 1980, the Wayne State University Department of Oncology conducted a phase II clinical trial of tamoxifen in disseminated malignant melanoma. The standard dose of tamoxifen, 10 mg orally twice a day, was used.

Twenty patients ranging in age from 34 to 81 years (median, 56) were entered in the study. Nine were female, 11 male. All but two patients had received previous chemotherapy or immunotherapy; most had been treated with multiple drugs, either dacarbazine alone or in combination. Disease-free interval ranged from zero (disseminated at diagnosis) to 8 years (median, 25 months). Median Karnofsky performance status at the time of entry into the protocol was 80 (range, 40–100). Two patients were unevaluable because of early death, defined as death within 2 weeks of entry into the study.

Of the 20 patients studied, 16 had predominantly soft tissue disease, three had lung disease, and one had lung and soft tissue disease.

Estrogen receptor protein was assayed in 12 of the 20 patients; three were not biopsied because the disease was primarily in the lung, one refused biopsy, and four had no readily assessable tumor. The results from one of the tumors assayed were invalid because of radioactivity in the sample. Of the 11 remaining assays, nine were negative for estrogen receptor protein and two were reported as "low": 3.6 and 4.0 fmols/10 mg wet weight of tissue.

Eighteen patients were evaluable for response (two were unevaluable because of early death). One patient had a partial response after 4 weeks of treatment, with regression of skin nodules. This response lasted for 3 weeks before the appearance of new disease. The estrogen receptor assay was negative in this patient. Toxic reactions were rare and consisted of mild nausea, weight gain, and hot flashes.

From the results of this study, we conclude that tamoxifen is not an effective agent in the treatment of disseminated malignant melanoma in patients who have received prior chemotherapy.

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[1] Received Dec 22, 1981; accepted Feb 2, 1982.

[2] Supported by contract N01-CM-07404 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Oncology, Wayne State University School of Medicine and Harper Hospital, Detroit, MI.

**Reprint requests to:* Michael K. Samson, MD, University Health Center, 4201 St. Antoine 7-C, Department of Oncology, Detroit, MI 48201.

LETTERS

Combination Antiemetics

Nausea and vomiting secondary to agents such as cisplatin are a major cause of morbidity in cancer chemotherapy (1). Gastrointestinal upset occurs in as many as 83% of these patients and may, in fact, be the limiting factor in chemotherapy (2). At the present time, only three major drug classes have been considered to be effective antiemetics: antihistamines, anticholinergics, and dopamine antagonists. These drugs are thought to act on the chemoreceptor trigger zone and/or emetic center in the medulla oblongata (2) where recent neuropharmacologic techniques have demonstrated the presence of histamine H_1 (3), muscarinic cholinergic (4), and dopamine (5) receptors. However, no single agent has yet proved to be effective in relieving severe nausea and vomiting. It thus follows logically that antiemetic efficacy might be improved by combining drug effects at all three proposed sites of action.

Morran et al (1) reported that the combination of fluphenazine and nortriptyline significantly reduced the incidence and severity of nausea and vomiting during cancer chemotherapy. The effectiveness of nortriptyline, an antidepressant, was thought to be related "to the relief of psychiatric morbidity which is common in patients receiving chemotherapy" (1). More likely, nortriptyline's antiemetic actions derive from its potent antihistamine and anticholinergic properties which have been documented in neurotransmitter receptor binding studies (6). In combination with fluphenazine, simultaneous blockade of histamine H_1, muscarinic cholinergic, and dopamine receptors occurs.

Other evidence also supports this hypothesis. Eight oncology patients at Stanford University Hospital were treated with prochlorperazine (10 mg iv every 4 hours) and diphenhydramine (25 mg iv every 4 hours) beginning 30 minutes prior to a 24-hour cisplatin infusion. Each patient had previously received between two and 11 cycles of cisplatin. Seven of the eight patients reported subjective preferences for the drug combination over any single agent used during prior chemotherapy cycles. The eighth patient denied any significant decrease in the amount of nausea and vomiting experienced during the cisplatin infusion. As with fluphenazine and nortriptyline, the simultaneous use of prochlorperazine and diphenhydramine results in blockade of dopamine, muscarinic cholinergic, and histamine H_1 receptors.

In summary, dopamine, cholinergic, and histamine antagonists all display antiemetic actions which are mediated via different pathways (2). Current neuropharmacologic techniques such as receptor binding assays provide a rapid and accurate measure of antiemetic effects at central nervous system receptor sites. It seems logical to employ these techniques to maximize antiemetic actions in the central emetic pathway. We are presently conducting a clinical trial to further investigate this hypothesis.

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Stephen J. Peroutka

Department of Neuroscience
The Johns Hopkins University
School of Medicine
725 N Wolfe St
Baltimore, MD 21205

January 18, 1982

Streptozocin-Induced Eosinophilia and Fever: A Case Report[1]

Streptozocin, a nitrosourea compound, has been used alone or with 5-FU to treat pancreatic islet cell carcinoma and carcinoid tumors (1–4). Its major toxic effects are renal and gastrointestinal. Uremia

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[1] Supported by a grant from the General Clinical Research Centers Branch (RR-53).

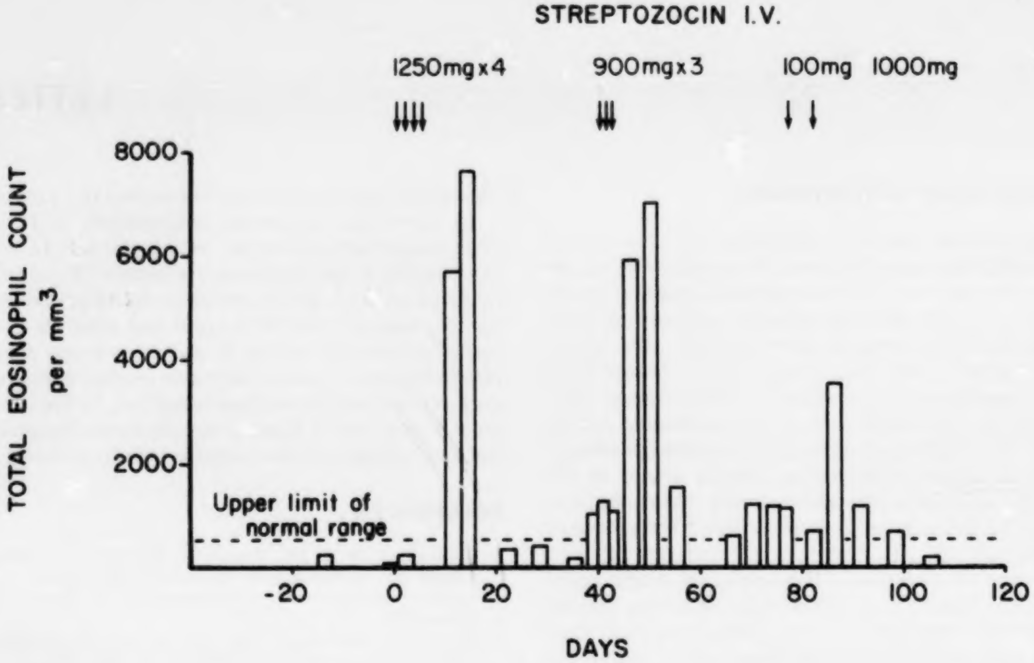

FIGURE 1.—Streptozocin-induced eosinophilia.

has occurred in a significant number of patients, and severe nausea and vomiting are common. Myelotoxicity occurs occasionally and hepatotoxicity has been reported in a few patients (1–4).

We are reporting the present case because of the relationship between streptozocin therapy and the development of fever, eosinophilia, and jaundice. Challenge with a lower dose of streptozocin reproduced fever and eosinophilia but not jaundice.

CASE REPORT

A 51-year-old man was admitted to the Clinical Research Center of Montefiore Hospital and Medical Center for the treatment of metastatic trabecular carcinoma of the skin. This tumor is believed to be a variant of carcinoid tumors arising in the skin (5). The primary malignancy was excised from the right buttock in June 1979 and regional node involvement was documented in April 1980. After initial radiation therapy, streptozocin and 5-FU therapy was instituted on July 9, 1979. The dose was 1250 mg/day of streptozocin and 900 mg/day of 5-FU for 4 days (fig 1). Prochlorperazine was administered to control vomiting. Ten days after completion of therapy, jaundice and marked peripheral blood eosinophilia were noted. The highest bilirubin was 3.9 mg/dl.

A second course of chemotherapy was instituted on August 18, 1979. The streptozocin dose was 900 mg and the 5-FU dose was 625 mg. This cycle was aborted after three doses because of fever, eosinophilia, and a bilirubin of 3.36 mg/dl. Rechallenge with a single dose of 100 mg of 5-FU followed 3 days later by 1000 mg did not produce side effects. Rechallenge with 100 mg of streptozocin was also uneventful, but a 1000-mg iv dose was followed 1 day later by a rectal temperature of 101.8°F and 3 days later by marked eosinophilia. Liver function abnormalities were not seen.

DISCUSSION

In our patient, hepatotoxicity and eosinophilia were noted 10 days after the first course of chemotherapy. In view of the multiplicity of drugs being used (5-FU, streptozocin, and prochlorperazine), it was not possible at first to implicate streptozocin as the causative agent. During the second course of therapy, the same side effects developed after only three doses of streptozocin and 5-FU without prochlorperazine. Although only fever and eosinophilia were reproduced after a single 1000-mg challenge dose of streptozocin, it is probable that jaundice would have recurred with the higher doses that were given in the first and second courses.

Hepatotoxicity is a known side effect of streptozocin therapy, although most investigators have found it difficult to separate liver function test abnormalities due to the disease from those due to the drug (1–4). Jaundice is rarely seen.

It appears that transient hepatic enzyme elevations occur frequently with streptozocin therapy, but that ours is the first reported patient who developed transient fever, eosinophilia, and bilirubinemia clearly related to streptozocin therapy. This combination of side effects strongly suggests an allergic reaction to streptozocin.

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Kumar A. Shah
Edward Greenwald*
Joseph Levin
Norman Rosen
Barnett Zumoff

Department of Oncology
The Clinical Research Center
Montefiore Hospital and
Medical Center
111 E 210th St
Bronx, NY 10467

February 1, 1982

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*To whom reprint requests should be sent.

ANNOUNCEMENTS

Update: Varicella Zoster Immune Globulin

Case by case review, which has been required for release of varicella zoster immune globulin (VZIG), is being suspended because distribution experience has shown that supply of this newly licensed product is adequate for all indicated uses. Initially there was little basis for projecting the potential demand following licensure; to insure that the supply would be adequate to meet the most critical requirements, the case by case review procedure was established. It is now evident that supply considerations will not require this review although any physician who is unfamiliar with the product indications or dosage is encouraged to seek the advice of staff physicians at the local distribution center.

VZIG produced by the Massachusetts Public Health Biologics Laboratories (MPHBL) and distributed by the American Red Cross Blood Services-Northeast Region (ARCBS-NR) is licensed by both the US and Canadian Bureaus of Biologics. It is made from plasma obtained by ARCBS-NR from healthy volunteer donors and is intended for passive immunization of susceptible immunodeficient individuals following exposure to chickenpox or zoster. Indications for use in those susceptible include congenital or acquired immunodeficiency, leukemia, lymphoma, or immunosuppressive therapy, combined with significant exposure to varicella or zoster patients. Use is also recommended for newborns whose mothers have onset of chickenpox less than 5 days before or 48 hours after delivery and for bone marrow transplant recipients regardless of previous varicella infection.

During the first year under FDA licensure, 6258 vials were distributed through regional depots established by ARCBS-NR in the US and Canada, 475 vials were distributed through MPHBL for use within the state of Massachusetts, and 297 vials were shipped overseas. Cooperative efforts between ARCBS-NR and MPHBL to identify and recruit normal volunteer donors with stable high-titer antibodies to varicella zoster virus have increased efficiency of production by lessening dependence on screening of random blood donations. The high demand months of winter and spring are upon us, and we look forward to being able to supply VZIG wherever the need arises.

For more information, contact: The American Red Cross Blood Services-Northeast Region (617) 449-0773.

Ions, Cell Proliferation, and Cancer

Lake Placid, New York, July 14–16, 1982

This symposium, sponsored by the National Cancer Institute and the W. Alton Jones Cell Science Center, will be held at the W. Alton Jones Cell Science Center, Lake Placid, New York. The focus will be on the roles of sodium, potassium, calcium, magnesium, pH, cyclic nucleotides, and calcium-binding proteins in a wide variety of proliferating systems and in cancer development. Techniques and methodology will also be discussed. The symposium co-chairmen are A. L. Boynton, W. L. McKeehan, and J. F. Whitfield.

For more information, contact: Dr. W. L. McKeehan, W. Alton Jones Cell Science Center, Old Barn Rd, Lake Placid, NY 12946.

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. Although teaching will be primarily provided through lectures, panel discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Fifth Annual Bristol-Myers Symposium on Cancer Research—Chromosomes and Cancer: From Molecules to Man

Chicago, Illinois, October 18–19, 1982

This symposium, sponsored by Bristol-Myers Company and to be held at the University of Chicago, will focus on the consistent chromosome abnormalities that occur in human tumors and will explore the use of new techniques that can elucidate the role of chromosome abnormalities in malignant transformation. Over 20 lectures will cover the following topics: chromosome structure, gene organization and control of gene expression; nonrandom chromosome aberrations in human leukemia and embryonic tumors; and the application of new techniques to determine the effect of chromosome abnormalities on gene function, including prophasing chromosomes, chromosome sorting, somatic cell hybrids, and in situ hybridization.

Speakers will include June Biedler, Anthony Carrano, Pierre Chambon, Geoffrey Cooper, Carlo Croce, H. John Evans, Uta Francke, Harvey Golomb, Ann Henderson, Ulrich Laemmli, Eric Lester, Felix Mitelman, Janet Rowley, Frank Ruddle, James Shapiro, Michael Sheffery, Ellen Soloman, Jack Spira, Hewson Swift, John Ultmann, David Ward, Harold Weintraub, Bryan Young, and Jorge Yunis.

For further information regarding registration, contact: Dr. Janet D. Rowley, or Dr. John E. Ultmann, The University of Chicago Cancer Research Center, Box 444, 950 E 59th St, Chicago, IL 60637.

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The Role of Intensive Postinduction Chemotherapy in the Management of Patients With Acute Myelogenous Leukemia[1]

Robert J. Mayer,* Howard J. Weinstein, Felice S. Coral, David S. Rosenthal, and Emil Frei, III [2]

During the past decade, combination anthracycline and cytarabine chemotherapy, sometimes accompanied by 6-thioguanine, has been associated with an increase in the complete response rate from 35%–55% (1,2) to approximately 75% (3–6) in patients < 60 years old having acute myelogenous leukemia (AML). This encouraging advance, however, has not been associated with prolonged periods of remission and indefinite survival in a significant number of patients, as have been seen in children with acute lymphocytic leukemia (ALL). Despite the use of "maintenance" chemotherapy, the median duration of remission in most recent investigations of AML is 12–15 months (3–14), with the long-term survivorship among such patients being 20%–25% (2,12,14,15). The frustrations encountered in attempting to improve these results have led some investigators to question the appropriateness of aggressive antileukemic therapy in terms of the human suffering caused by such treatment (16), thereby implying that AML is a basically incurable disorder.

Before accepting the inevitability of relapse in the patient with AML, it seems wise to reassess the meaning of maintenance therapy in this disease and to determine whether past therapeutic efforts have adequately addressed the problem of eradicating clinically undetectable residual blast cells during remission.

Maintenance Therapy in Childhood ALL

On the basis of postmortem analysis and cell-cycle time determinations, the leukemic cell burden in a child with ALL has been estimated to be in the range of 1.0×10^{12} cells (17). Treatment with chemotherapy leading to the achievement of a complete remission is thought to result in a 1000–10,000-fold reduction in the number of leukemic cells (17). The discontinuation of drug treatment immediately after remission induction leaves the patient with the clinically undetectable presence of 10^8–10^9 leukemic cells, making subsequent relapse a near certainty. Clinical experience has offered support for this concept. A prospective trial performed 20 years ago in which children with ALL in remission were randomized to 6-mercaptopurine maintenance therapy or a placebo clearly demonstrated that additional drug treatment leads to an increased duration of remission (18). The introduction of maintenance therapy, particularly drug combinations, to further reduce and eliminate residual leukemic cells has been one of the fundamental steps leading to the long-term disease-free state presently attained by most children with this disease (19).

The acute toxicity when children are treated for ALL is far milder than that observed with therapy for AML. Complete remission in childhood ALL can generally be achieved without the development of marrow aplasia. Lymphoblasts are extremely sensitive to relatively nonmyelosuppressive agents (eg, vincristine, corticosteroids, and L-asparaginase) as well as to drugs that damage bone marrow elements (eg, methotrexate, 6-mercaptopurine, and anthracyclines). Since the chemotherapeutic agents employed in the treatment of childhood ALL have been associated with "acceptable" side effects and a gratifying therapeutic outcome, there has been no reluctance to continue the use of maintenance therapy in this disease.

Should the Therapeutic Principles Utilized in ALL be Applied to AML?

Whereas ALL appears to be a neoplasm that has infiltrated the bone marrow, AML represents a cytogenetically definable clonal expansion (20,21) of abnormal hematopoietic cells having a maturation defect at one of several possible sites in the differentiation pathway emanating from the pluripotent stem cell (22). In contrast to the situation in ALL, leukemic myeloblasts are only moderately sensitive to chemotherapy. Complete remissions in AML can usually be achieved only through the use of inten-

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[1] Received Mar 8, 1982; accepted Mar 9, 1982.

[2] Divisions of Medical and Pediatric Oncology, Sidney Farber Cancer Institute; Division of Hematology, Brigham and Women's Hospital; and Departments of Medicine and Pediatrics, Harvard Medical School, Boston, MA.

**Reprint requests to:* Robert J. Mayer, MD, Division of Medical Oncology, Sidney Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

sive drug treatment leading to bone marrow aplasia. Therapeutic strategy is based on the premise that normal marrow elements will recover more rapidly than abnormal, leukemic clones. The recent improvement in the remission-induction rate in patients with AML (3–6) is a reflection of higher, more intensive doses of chemotherapy as well as the added availability of broader-spectrum antibiotics and platelets for transfusion to "support" such individuals through periods of extreme myelosuppression.

There is every reason to believe that patients with AML in complete remission harbor a similar leukemic cell burden (ie, 10^8–10^9 cells) as has been estimated for children with ALL (17). In keeping with the experience in ALL, patients with AML in complete remission may be expected to relapse within several months if no further treatment is administered. It would furthermore seem reasonable that continued (ie, maintenance) chemotherapy of the type that led to complete remission should be effective in extending the period of remission by further decreasing the number of residual leukemic cells. Such treatment would seem essential if the goal in the treatment of patients with AML is not palliation but cure.

Does Maintenance Therapy Prolong Remission in AML?

It is difficult to compare published data reporting the duration of remission in patients with AML. Most studies are limited by their small patient size and short followup times. Perhaps the greatest impediment to such comparisons is the variety of remission-induction regimens utilized prior to patient evaluation during the postremission period. Drug treatment plans associated with a high remission-induction rate may quite possibly be more intensive, thereby leading to a greater "leukemic cell kill," resulting in a longer period of remission without maintenance therapy. In contrast, somewhat less intensive induction regimens followed by relatively low-dose maintenance plans may well lead to a total remission duration equal to that achieved by quite intensive induction programs alone.

The experience accumulated in seven series (8,9,11,13,23–25) in which AML patients in complete remission were given no maintenance therapy is outlined in table 1. Four of these patient groups (8,9,11,13) received several courses of "consolidation" treatment after remission was achieved, while the other three (23–25) were given no postinduction therapy. The complete remission rate in these clinical trials ranged from 40% (23) to 60% (13), reflecting in large part the intensity of the induction regimen. The median duration of remission for these unmaintained patients varied from 2.0 months (23) to 9.5 months (25), again a measure of the dose and schedule of the induction treatment. Regardless of the induction plan utilized, > 50% of these unmaintained patients relapsed within 10 months. A 1-year remission duration > 35% was reported in only one study (25). This clinical trial, performed by investigators at Johns Hopkins University, explored the effect of timed-sequential chemotherapy and was so intensive that marrow recovery in responding patients was not observed until almost 4 weeks after the start of treatment (25). While the probability of achieving a second remission may be increased in unmaintained patients (25), presumably through a reduction in the development of drug resistance,

TABLE 1.—Comparative published results: remission duration without maintenance therapy

Therapy*		Complete remission (%)	No. of patients in remission without chemotherapy	Median duration of complete remission (mos)	% in remission at 1 yr	Ref No.
Remission induction	Consolidation					
DNR or POMP	None	40	17	2.0	12	23
Ara-C, 6-TG	Ara-C, 6-TG (3 mos)	41	24	5.3	10	9
DNR. Ara-C, 6-TG†	DNR, Ara-C, 6-TG (2 mos)	51	37‡	6.7	Not stated	11
DNR, Ara-C, 6-TG†	Ara-C, 6-TG (1 mo)	Not stated	13	6.7	0	8
DNR, Ara-C, 6-TG, ADR, Cyclo	ADR, Cyclo	60	15§	8.0	33	13
Cyclo, Ara-C, VCR	None	46	12	8.5	25	24
DNR, Ara-C	None	56	19	9.5	47	25

*DNR = daunorubicin; POMP = prednisone, vincristine, methotrexate, and 6-mercaptopurine; Ara-C = cytarabine; 6-TG = 6-thioguanine; ADR = doxorubicin; Cyclo = cyclophosphamide; VCR = vincristine.

†Part of randomized study vs maintenance chemotherapy.

‡18 of these patients received sc injections of BCG.

§Received BCG + irradiated allogeneic AML cells.

second remissions in nontransplanted patients with AML are of short duration and survival generally parallels the duration of the first remission.

The results of 11 representative studies (2,3,8–13,26–28) in which adults and children with AML in complete remission were given maintenance chemotherapy are detailed in table 2. In contrast to the observation in the unmaintained groups, nine of the ten maintenance trials in which sufficient data are available report that 35% of the patients have remained in continuous remission after 1 year. The four treatment plans having the highest remission-induction rates are associated with the longest median remission durations (> 12 months) and percentages of patients remaining in complete remission after 12 months (> 50%) (3,12,27,28). The maintenance programs in these four studies varied in intensity.

The data from tables 1 and 2 strongly suggest that the use of maintenance chemotherapy will prolong remission duration in patients with AML. This conclusion is supported by a prospective randomized study (8) in which five of 13 (38%) patients receiving modest doses of cytarabine and 6-thioguanine remained in continuous complete remission after 12 months, while none of 13 similar unmaintained patients remained disease-free. A second prospectively randomized study failed to show an advantage for the use of a relatively inactive drug (carmustine [BCNU]) in combination with a single monthly injection of cytarabine in the maintenance arm (11).

TABLE 2.—Comparative published results: remission duration with maintenance therapy

Therapy*			Complete remission (%)	No. of patients in remission	Median duration of complete remission (mos)	% in remission at 1 yr	Ref No.
Remission induction	Consolidation	Maintenance					
Ara-C, 6-TG	Ara-C, 6-TG (3 mos)	DNR, 6-TG, Cyclo, VCR, BCNU, hydrea (L-6 regimen)	41	24	6.0	39	9
DNR, Ara-C, 6-TG†	DNR, Ara-C, 6-TG (2 mos)	Ara-C, BCNU	51	17	7.0	Not stated	11
DNR, Ara-C, 6-TG, ADR, Cyclo	ADR, Cyclo	DNR, Ara-C, 6-TG	60	17‡	8.0	33	13
ADR, Ara-C, VCR, Pred	ADR, Ara-C, VCR, Pred	DNR, Ara-C, 6-TG	45	39§	8.0	42	10
Ara-C, 6-TG	Ara-C, 6-TG	DNR, 6-TG, Cyclo, VCR, BCNU, hydrea, MTX (L-6 regimen)	56	49	10.0	45	2
DNR, Ara-C, 6-TG†	Ara-C, 6-TG (1 mo)	Ara-C, 6-TG (monthly)	Not stated	13	10.3	38	8
Ara-C, 6-TG, Cyclo, Pred, VCR	None	Ara-C, 6-TG, Cyclo, VCR	59	94	11.5	48	26
DNR, Ara-C, 6-TG	DNR, Ara-C, 6-TG	DNR, Ara-C, 6-TG (24-mo duration)	82	56	13.0	55	3
DNR, Ara-C or DNR, Pred or Ara-C, 6-TG	None	Ara-C, 6-TG (60-mo duration)	58	26	17.0	52	12
ADR, Ara-C, VCR, Pred	None	ADR, Ara-C, azacitidine, MTX, 6-MP, Pred, VCR (VAPA-10 regimen—16-mo duration)	70	58	23.0	67	27
ADR or DNR, Ara-C	ADR, Ara-C, Cyclo (3 mos)	ADR, Ara-C, 6-TG, Cyclo, methyl-GAG (970701 regimen)	90	20	37.0	69	28

*Ara-C = cytarabine; 6-TG = 6-thioguanine; DNR = daunorubicin; Cyclo = cyclophosphamide; VCR = vincristine; BCNU = carmustine; hydrea = hydroxyurea; ADR = doxorubicin; Pred = prednisolone; MTX = methotrexate; 6-MP = 6-mercaptopurine.

†Part of randomized study vs no maintenance therapy.

‡Also received BCG + irradiated allogeneic AML cells.

§Also received BCG.

Is There a Need for Intensive Postremission Therapy in AML?

The traditional definition of the term "maintenance therapy" is that of a relatively mild continuation of treatment, having the purpose of "maintaining" complete remission without placing the patient at risk for undue toxic reactions. In large part, this concept evolved from the experience with childhood ALL in which such an approach was quite successful. Inserting the same principle into the management of patients with AML, while resulting in somewhat longer remissions than those in unmaintained groups, has not led to median remission durations > 12–15 months. One of the possible explanations for this observation, as noted previously, is the decreased sensitivity of the leukemic myeloblast to chemotherapy. If high-dose treatment resulting in marrow aplasia is required to induce a complete remission in AML, it seems highly unlikely that the number of residual leukemic cells will be further reduced or even stabilized by the use of relatively nonmyelosuppressive, intermittent, maintenance treatment.

Because of the narrow therapeutic index of cytotoxic agents in the treatment of AML, the drug doses required to kill leukemic myeloblasts during remission induction severely suppress normal hematopoietic progenitors, leading to a period of pancytopenia. It is reasonable to assume that similar myelosuppressive treatment will be necessary after remission is achieved to further reduce the leukemic cell burden. The use of such intensive therapy during remission, even at doses greater than those administered during induction, takes advantage of the steep dose-response curve that has been demonstrated for chemotherapeutic agents such as cytarabine in experimental tumors (29) and in human leukemic cells in tissue culture (30). A linear-logarithmic relationship between dose and reduction of tumor cells has been shown in these systems (29,30). The administration of such high-dose maintenance treatment is also consistent with the proposal that intensification may be necessary to eradicate leukemia at a time (ie, remission) when the leukemic cell burden has been reduced (31).

Two recent clinical trials (27,28) (included in table 2) have utilized the principle of intensive maintenance therapy in groups of young patients. One study (27), from the Sidney Farber Cancer Institute and known as VAPA, included 16 courses of sequential chemotherapy, with cytarabine being given in eight of these courses by continuous administration at a dose (200 mg/m²/day) higher than that used during remission induction. The second trial (28), conducted at Roswell Park Memorial Institute, offered patients three courses of high-dose consolidation treatment followed by a sequential maintenance program. The median duration of remission and the percent of patients remaining in remission after 12 months in these studies are higher than those noted in previous investigations (table 2).

The VAPA Experience: An Update

A total of 107 consecutive previously untreated patients < 50 years of age with AML were entered in this study between February 1976 and May 1980. The experimental design and the preliminary results in the first 83 patients have been reported previously (27). The median age for the entire group

TABLE 3.—Update of VAPA experience

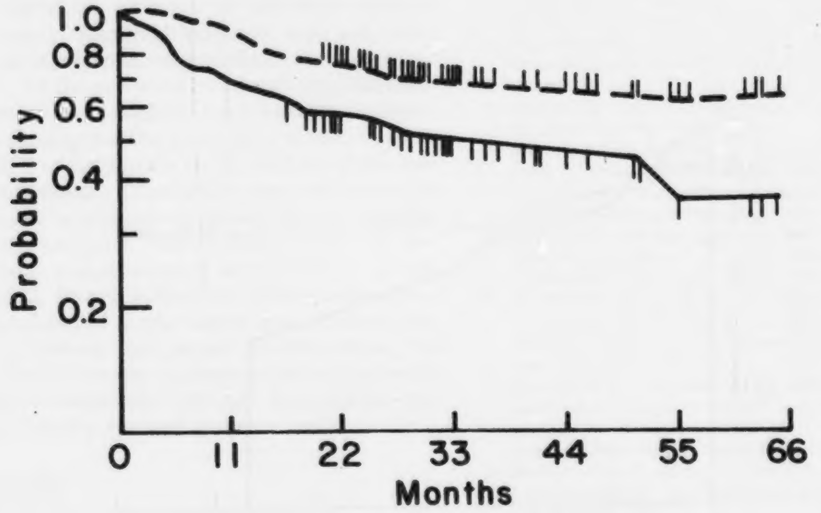
	No. of patients by age		
	0–50 yrs (median, 14.5)	0–17 yrs (median, 9.5)	18–50 yrs (median, 34.5)
Entered in study	107	61	46
Complete remissions	75 (70%)	45 (74%)	30 (65%)
Withdrawals	8	5	3
Deaths during remission	2	0	2
Relapses	32	18	14
Bone marrow	21	9	12
CNS	9	8	1
Myeloblastoma	2	1	1
Continuous complete remission	33	22	11
Completed therapy	37	25	12
Relapses while not receiving therapy	8	4	4

was 14.5 years, reflecting the pattern of patient referral. An analysis of this experience as of October 1, 1981, is shown in table 3. Seventy-five of the 107 patients (70%) achieved complete remission with an induction program that included 3 days of an anthracycline (doxorubicin) and 7 days of continuous-infusion cytarabine (100 mg/m²/day) with the addition of vincristine and prednisolone. The patients who entered complete remission were then treated with intensive chemotherapy during a 14–15-month maintenance period, with the treatment subdivided into four sequences of drug combinations (table 4). Such intensive treatment was frequently associated with the development of severe thrombocytopenia requiring platelet transfusions and neutropenia accompanied by fevers, necessitating short-term hospitalizations and the use of broad-spectrum antibiotics.

The median duration of followup time for the 75 patients who entered complete remission is now 19 months, and only three patients are still receiving protocol treatment. There have been 32 relapses,

TABLE 4.—VAPA: intensive sequential maintenance schema

Sequence	Drug regimen
I	Doxorubicin, 45 mg/m²/day, Day 1, iv pulse Cytarabine, 200 mg/m²/day, Days 1–5, continuous infusion q3–4 wks × 4
II	Doxorubicin, 30 mg/m²/day, Day 1, iv pulse Azacitidine, 150 mg/m²/day, Days 1–5, continuous infusion q4 wks × 4
III	Vincristine, 1.5 mg/m²/day, Day 1, iv pulse Methylprednisolone, 800 mg/m²/day, Days 1–5, iv pulse 6-Mercaptopurine, 500 mg/m²/day, Days 1–5, iv pulse Methotrexate, 7.5 mg/m²/day, Days 1–5, iv pulse q3 wks × 4
IV	Cytarabine, 200 mg/m²/day, Days 1–5, continuous infusion q3 wks × 4



– – Survival:	Alive	Dead	Total	Median
	48	27	75	undefined
— Continuous Remission:	Continuous CR	Relapse	Total	Median
	41	34	75	31.9 months

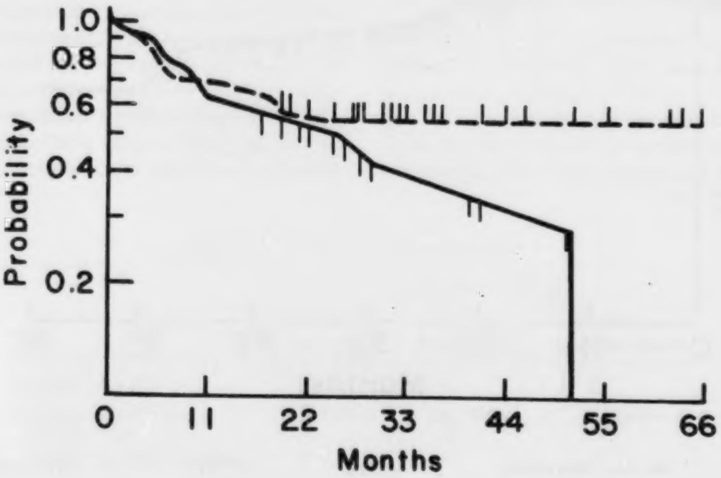
FIGURE 1.—VAPA: Kaplan-Meier plot of probability of survival and probability of remaining in complete remission among all complete responders. Ticks indicate the 40 patients who remain alive and the 33 patients who continue in complete remission. The 8 patients who were "withdrawn" from the study contributed to the curves until the time of their withdrawal.

two deaths during remission, and eight withdrawals during remission. Thirty-three patients remain in continuous complete remission, with the median followup time for this subset being 31 months. Of 37 patients who have completed the sequential maintenance regimen, eight have relapsed after completing therapy while 29 remain in "unmaintained" complete remission. The median duration of followup time after the completion of therapy for this group of 37 patients is 14 months (range, 1+–50+).

Figure 1 presents a Kaplan-Meier plot of the probability of continued complete remission and survival among the patients achieving a complete response. As previously noted, treatment was discontinued at approximately 15 months. The median duration of complete remission is estimated to be 31.9 months, while the median duration of survival is undefined. Utilizing two standard deviation confidence limits (2 SD), the probability for patients achieving a complete response to remain in remission after 12, 24, and 36 months is 68% ± 11%, 55% ± 13%, and 51% ± 18%, respectively.

Figure 2 presents a Kaplan-Meier plot comparing the duration of complete remission in the pediatric and adult subgroups. The median remission duration in the pediatric group is undefined. with 55% ± 13% (2 SD) of patients remaining disease-free at 24 months and no child having relapsed after that time. In the adult group, 50% ± 25% (2 SD) of patients are estimated to remain in complete remission at 26 months. The relatively wide range in 2 SD confidence limits for the adults is a reflection of the small number of these patients in this trial. Additionally, the adult curve is strongly influenced by the development of two late relapses that occurred in a 40-year-old woman and a 21-year-old man, both of whom had completed therapy 36 months previously. The total duration of remission for these two patients was 51 and 55 months, respectively. Such late failures, while unusual, have been observed by others (32).

Reanalysis of the results of the VAPA program, therefore, continue to support the use of intensive postinduction therapy for patients with AML, especially in the pediatric age group. These data furthermore suggest that chemotherapy alone can effectively suppress the leukemic clone for extended periods of time and raise the possibility that a significant number of these patients may be cured of their disease.



Age	Continuous CR	Relapse	Total	Median
– – – 0-17 years	22	18	40	undefined
—— 18-50 years	11	16	27	25.6

FIGURE 2.—VAPA: Kaplan-Meier plot of probability of remaining in complete remission, comparing pediatric and adult age groups. Ticks indicate the 22 children and 11 adults who continue in complete remission. The 5 children and 3 adults who were "withdrawn" from the study contributed to the curves until the time of their withdrawal.

Allogeneic Bone Marrow Transplantation: Another Approach to Intensive Maintenance Therapy

Because of the increasing evidence that AML is a clonal disease, possibly representing a malignancy of the pluripotent stem cell (22), and because of the short remission duration associated with nonintensive maintenance chemotherapy, several investigators have directed their efforts at replacing the stem cell pool during first remission through the use of ablative chemotherapy and radiation therapy followed by allogeneic bone marrow transplantation. The initial experience with this form of therapy reported from several transplantation centers (33–35) in patients generally < 35 years of age has been encouraging, with graft-versus-host disease representing the major cause of morbidity and with leukemic relapse being relatively uncommon. Approximately 60% of patients with AML who undergo bone marrow transplantation during their first remission may be expected to be alive 24 months after the graft, and failure thereafter has been uncommon.

In a sense, allogeneic bone marrow transplantation represents the ultimate in "intensive maintenance" therapy. Residual leukemic cells are eliminated by ablative treatment and the stem cell pool is replaced. At the present time, however, this therapeutic option is limited by immunologic considerations. Eligibility for the procedure is restricted to the 30%–40% of patients who have an available histocompatible donor. Even when marrow from such an ideal donor is utilized, graft-versus-host disease will be observed after 50% of transplants (36) and will be a fatal complication in about 33% of the patients (35). A future reduction in the incidence of the chronic form of graft-versus-host disease may result in a limited therapeutic benefit, since this "complication" appears to produce an antileukemic effect (36), presumably through lymphocyte-mediated cytotoxicity toward leukemic cells.

CONCLUSION

The results of aggressive approaches, such as high-dose sequential chemotherapy (eg, the VAPA program) and allogeneic bone marrow transplantation following marrow ablation, indicate that intensive treatment for patients with AML in remission can lead to a prolonged "unmaintained" disease-free state. The comparative merits of these two treatment modalities have been the focus of debate (37).

Patients with AML who receive some form of maintenance chemotherapy remain in complete remission longer than those who do not receive such treatment. The VAPA program and the Roswell Park experience suggest that intensive postinduction treatment may lead to longer durations of remission. Interpretation of these data, however, should take into account the young median ages in both studies. In addition, such issues as the intensity and duration of postinduction chemotherapy remain unsettled. Furthermore, it has yet to be shown in a prospectively randomized fashion that intensive postremission therapy in patients with AML is superior to regimens with less myelosuppressive treatment. Future clinical trials should directly address these issues before any definite conclusions can be made.

At the present time, the emphasis for physicians who treat patients with AML should no longer be limited to a goal of remission induction but should now be expanded to aggressive means of prolonging that remission and achieving a cure.

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Marrow Transplantation for Patients With Acute Nonlymphoblastic Leukemia Who Achieve a First Remission[1,2]

E. Donnall Thomas,* Reginald A. Clift, and C. Dean Buckner[3]

For the Seattle Marrow Transplant Team

Marrow transplantation for patients with acute nonlymphoblastic leukemia (ANL) was first carried out for patients in relapse, most of whom had failed to respond to combination chemotherapy. In 1977 we reported a series of 54 such patients given 60 mg/kg of cyclophosphamide on each of 2 days, followed by 1000 rad of total-body irradiation and marrow grafts from HLA-identical siblings (1). There were many deaths in the first weeks after grafting, due to the complications of advanced illness, graft-versus-host disease, and opportunistic infections. Sixteen patients had a recurrence of leukemia in subsequent months, with the last relapse of leukemia occurring 18 months after transplantation. An actuarial analysis shows that 61% of these patients would have had a relapse of leukemia if there were no other causes of death (fig 1). Six of these patients, who received no antileukemic therapy after grafting, are alive in continuing remission and leading essentially normal lives, now 6–10 years after transplantation. These patients ranged in age from 16 to 36 years at the time of transplantation. Three have had cataract operations and none have chronic graft-versus-host disease. Figure 1 shows the survival curve for these 54 patients and demonstrates that the survivors are far out on a plateau and presumably cured of the disease (2).

In view of the apparent cure of a small fraction of end-stage patients, we turned to transplantation in first remission for patients with ANL (3). The regimen consisted of 60 mg/kg of cyclophosphamide × 2 and 920 rad of total-body irradiation, followed by marrow from an HLA-identical sibling. Ten of the first 19 such patients, reported in 1979, are now living in remission. Three additional patients were treated with this regimen and two are in remission. The median disease-free survival has not been reached but will be ≥ 44 months from transplantation.

Our next study was designed to evaluate the possibility of improving results by the use of fractionated total-body irradiation (4). Fifty-three patients with ANL in first remission were treated with cyclophosphamide, total-body irradiation, marrow transplantation, and the usual regimen of methotrexate given in the first 100 days after transplantation. Twenty-seven patients were randomized to receive 1000 rad in one exposure, and 26 were given 200 rad on each of 6 days. Eleven of the patients given the single exposure and 15 of the fractionated irradiation recipients are living.

Thus, 75 patients with ANL have been transplanted in first remission, with the survivors followed for 12–58 months. Only six patients have had a recurrence of leukemia. The median time from transplant to relapse was 12 months (range, 6–30). Five of the six relapses occurred within 14 months of transplant. Figure 2 shows a Kaplan-Meier plot (5) of survival and of the probability of being in remission for these 75 patients. One patient died of a pneumothorax while in remission 4.5 years after grafting.

In our studies, as well as in the studies of other marrow transplant teams, emphasis has been placed on transplanting younger patients. Our transplant protocols provide an upper limit of 50 years of age. Figure 3 shows the Kaplan-Meier plot of the probability of survival according to age for the 75 patients with ANL in first remission described above. The fraction of long-term survivors is greater for the younger patients ($P = 0.03$), and the difference in survival appears to be almost entirely related to the increased frequency and severity of graft-versus-host disease in the older patients.

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[1] Received Feb 8, 1982; accepted Feb 9, 1982.

[2] Supported by Public Health Service grants CA-18029, CA-15704, and CA-18579 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Thomas is the recipient of Research Career Award AI-02425 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services.

[3] Fred Hutchinson Cancer Research Center and the University of Washington School of Medicine, Seattle.

**Reprint requests to:* E. Donnall Thomas, MD, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA 98104.

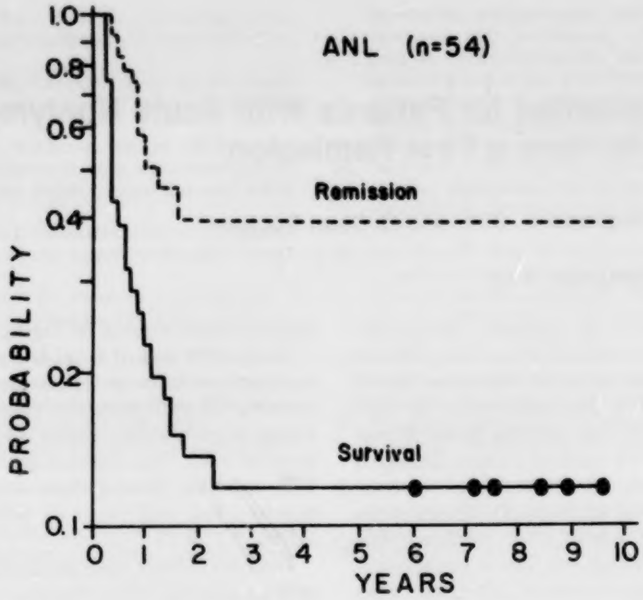

FIGURE 1.—Kaplan-Meier product limit estimates of the probability of survival and of being in remission for 54 patients with ANL who had transplants in relapse. Dots indicate 6 patients living in remission.

DISCUSSION

The actual (not projected) 3-year survival was 59% for the first 22 patients transplanted while in remission described above. The median survival from transplantation will be ≥ 44 months, and the median time from initial diagnosis and treatment will be > 47 months. These figures are superior to any yet reported for patients treated with combination chemotherapy. The actuarial analysis of the subsequent 53 patients and the reports by two other marrow transplant teams (6,7) provide additional evidence of the superiority of the marrow transplant regimens.

A comparison of median survival data is informative but not definitive. The major question is how many patients are actually being cured. The apparent cure of end-stage patients with marrow transplantation, now borne out by 6–10 years of followup, indicates that many of the patients transplanted in first remission are likely to be cured (fig 2). The actuarial analysis indicates that marrow transplantation for patients between 20 and 30 years of age with ANL in first remission will cure slightly more than one half of the patients. For patients < 20 years old, the cure rate will be superior, and for those > 30 years old, the results will be worse (fig 3).

Marrow transplantation is presently the treatment of choice for selected groups of patients only. First of all, the patient must have a suitable marrow donor. Our experience indicates that approximately 40% of the patients will have HLA-identical siblings. Current exploration of transplants using haploidentical family-member donors (8) and unrelated phenotypically HLA-identical donors (9) is encouraging and points the way to a much larger number of potential candidates for marrow transplantation. Second, age is an important factor in considering marrow transplantation for patients with ANL in first remission. Marrow transplantation is currently limited to patients < 50 years old, and the most beneficial results are achieved with patients < 30 years old.

Several marrow transplant centers are conducting studies designed to improve the results of marrow transplantation, including regimens of fractionated irradiation, cyclosporin A, interferon, and monoclonal antibodies. Similarly, attempts are being made to improve the results of combination chemotherapy by the use of new drugs, drug combinations, and immunotherapy. Claims of cure by any

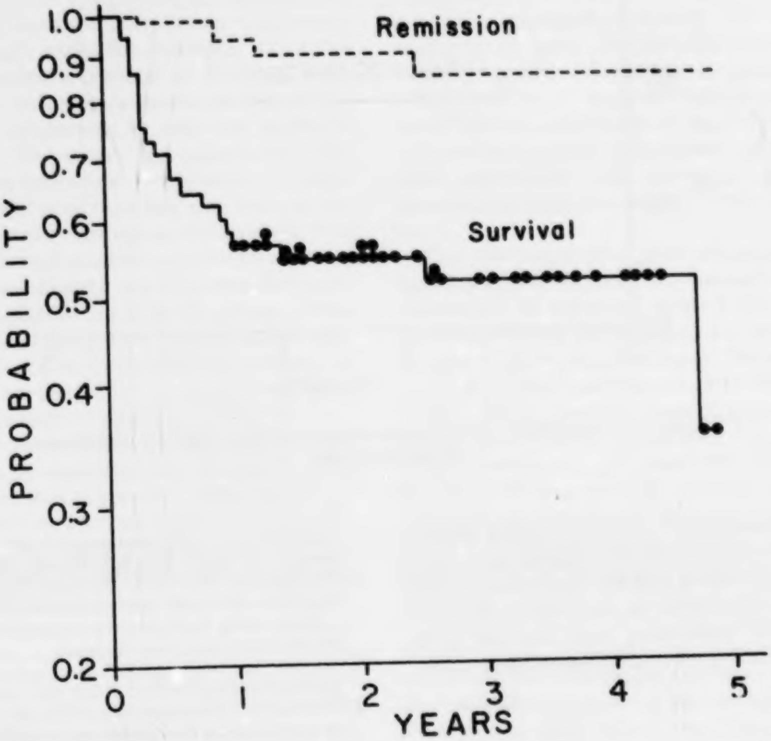

FIGURE 2.—Kaplan-Meier product limit estimates of the probability of survival and of being in remission for 75 patients with ANL who had transplants in 1st remission. Dots indicate 38 patients living in remission.

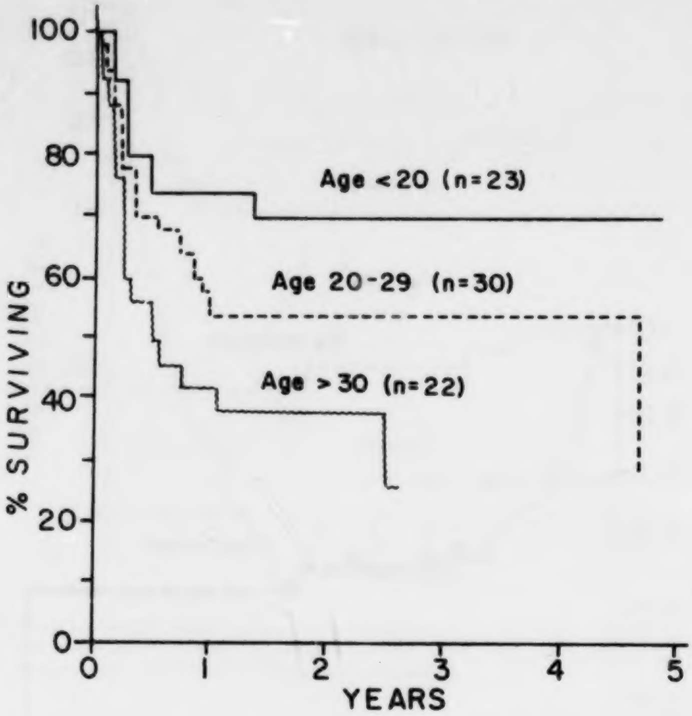

FIGURE 3.—Kaplan-Meier product limit estimates of the probability of survival according to age.

therapeutic regimen must be based on the demonstration of long-term survival after therapy is stopped and on the demonstration of a flat plateau of several years' duration on an actuarial analysis of the probability of being in remission, most evident when these data are plotted on a semilogarithmic scale.

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Therapy for Patients With Acute Myelocytic Leukemia Who Enter Remission: Bone Marrow Transplantation or Chemotherapy?[1,2]

H. D. Preisler[3,*]

Substantial progress has been made in the treatment of young patients with acute myelocytic leukemia. Remission rates for individuals < 30 years of age are very high with, for example, 42 of the last 45 of such patients treated at Roswell Park Memorial Institute entering complete remission (1), a remission rate comparable to that for pediatric acute lymphocytic leukemia. The papers by Drs. Mayer and Thomas and their co-workers (1,2) suggest that substantial progress has also been made with respect to remission duration and survival. The marrow transplantation studies reported by Blume et al (4) and Powles et al (5) provide data that confirm the early results of the Seattle group, while studies of the role of intensive consolidation chemotherapy carried out at Roswell Park provide remission duration curves that are similar to those reported by Mayer and colleagues (1) (fig 1).

A side-by-side comparison of the Mayer and Thomas papers (1,2) suggests both similarities and differences in treatment outcomes. Both studies report the treatment results for 75–80 patients followed for 1–5 years. Comparison of the survival curves and the remission duration curves suggests that significant differences may emerge in the future. The proportion of surviving patients appears to be greater for the chemotherapy study at all points in time. The differences in the slopes of the curves are greatest for the first year, a reflection of the high initial mortality rate for the transplanted patients. In contrast, examination of the relapse curves suggests an advantage for the transplantation approach, since the chemotherapy curve indicates a continuous relapse rate for the duration of the study while the comparable curve for transplanted patients appears to be relatively flat with few relapses. These data reflect the fact that the problems associated with each therapeutic approach are different, with the transplantation studies essentially limited by treatment-related deaths and the chemotherapy approaches limited by the problem of leukemic relapse. Partial solutions of these problems will probably result in a merging of the problems, with more aggressive chemotherapy associated with an increase in disease-free mortality and a decrease in graft-versus-host (GVH) disease perhaps associated with leukemic relapse in transplanted patients becoming more common (see below).

The likelihood of a good therapeutic outcome is age-dependent for both therapeutic approaches. For patients < 18 years old treated with chemotherapy, the relapse-free curve appears to be flat, suggesting a state free of risk of relapse. There are, however, only 22 patients on this curve so that the relapse of even a single patient could significantly alter the shape of the curve. Similarly, the best results for transplantation are for patients who are < 20 years of age, with the survival of patients > 30 years old being projected at only 20%–40% at 2½ years.

A potential advantage for the chemotherapeutic approach is that therapeutic failure currently means leukemic relapse, a treatable event. In contrast, treatment failure for a transplant patient usually means death. Although patients who relapse while receiving chemotherapy probably cannot be cured by currently available chemotherapeutic agents, the Intergroup AML study has found that approximately 50% of these patients will re-enter remission after therapy with a cytarabine/doxorubicin antibiotic regimen and that the current median duration of unmaintained remission is 4¼ months (6). One would expect that patients who relapse after therapy is stopped [as have one half of the relapsers in the Mayer et al study (1)] will be even more likely to re-enter remission and to have a longer duration of remission if they receive consolidation therapy. Therefore, even though the chemotherapy patients appear to be on a continuous relapse curve, the survival curve of the patients who were the subjects of the Mayer report will not necessarily cross that of the transplanted patients because of the likelihood that new approaches to chemotherapy will be available in the future.

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[1] Received Mar 8, 1982; accepted Mar 9, 1982.

[2] Supported by Public Health Service grant CA-5834 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Roswell Park Memorial Institute, Buffalo, NY.

[*] *Reprint requests to:* Harvey D. Preisler, MD, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263.

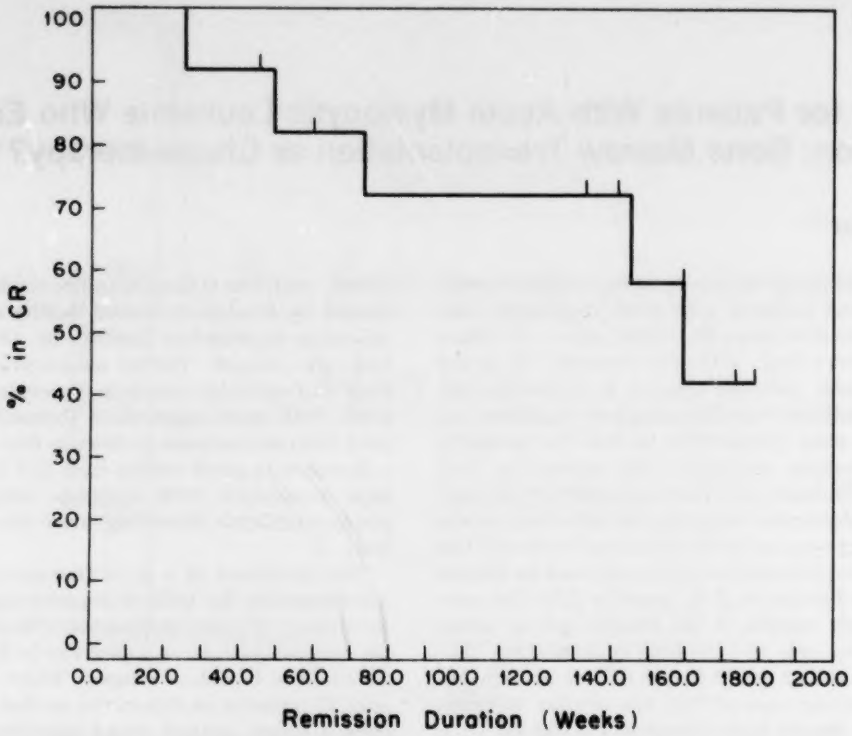

FIGURE 1.—Remission duration curve for patients < 30 yrs of age treated with intensive consolidation chemotnerapy. Patients were induced into remission with cytarabine-doxorubicin. Consolidation therapy consisted of: *course 1*—100 mg/m^2 of cytarabine twice a day × 6 days and 30 mg/m^2/day of doxorubicin × 2; *course 2*—100 mg/m^2 of cytarabine twice a day × 7 days and 500 mg/m^2 of cyclophosphamide on Day 1; *course 3*—100 mg/m^2/day of cytarabine by continuous infusion × 7 days and 30 mg/m^2/day of doxorubicin × 3. Once consolidation was completed, patients received daily 6-thioguanine (40–80 mg/day) and weekly methyl-GAG (300 mg/m^2). Every 3 mos they received consolidation chemotherapy consisting of an alternation of courses 2 and 3 for 3 yrs.

SPECIFIC COMMENTS ON BONE MARROW TRANSPLANTATION IN FIRST REMISSION

The use of marrow allografting after ablative therapy has several theoretic advantages over the chemotherapeutic approach. In principle, if one could deliver truly ablative therapy without having to leave enough normal hematopoietic stem cells to repopulate the patient's bone marrow, then one could attempt to kill the "last" leukemic cell. As will be discussed below, it is difficult to imagine such definitive therapy being accomplished by the currently available chemotherapeutic agents. Replacement of a patient's hematopoietic stem cells would be particularly important if, as has been speculated, there are no normal residual progenitor cells in leukemic patients (7). Unfortunately, as described below, it does not appear likely that even the currently employed ablative transplant preparative regimens are capable of destroying the "last" leukemic cell. The use of total-body irradiation as part of the transplant preparative regimens has a potential advantage over chemotherapy in that radiation therapy reaches all sanctuary sites independent of various "barriers" that affect drug distribution. This may be particularly important in preventing late relapses, since these appear to be occurring in unusual sites such as the skin, central nervous system, etc—problems reminiscent of pediatric acute lymphocytic leukemia. Since it appears likely that identical twin transplantation is inferior to allogeneic grafting in terms of antileukemic efficacy, despite the same preparative regimens being used, the long-term disease-free survival associated with allografting must be the result of some additional phenomena.

A claimed advantage of transplantation is that the therapy is administered once and not in repeated courses as is consolidation chemotherapy. This claim must be viewed, however, with some degree of skepticism, since it is likely that GVH disease plays a role in maintaining the transplanted patients in a state in which leukemia is at least not clinically apparent (8,9). Hence, it appears that one of the antileukemia components of transplantation involves a process that persists for a long period of time and that in and of itself may produce late and unexpected toxic effects. Since only a small number of patients have actually been followed for more than several years, there are essentially no data regarding long-term complications, which may include damage to vital organs, second malignancies, and premature aging. The long-term and continuous nature of GVH disease also suggests that patients in long-term remission may not be disease-free but rather that leukemic cells may be present at a subclinical state, with the inherent possibility of late relapse. While the initial experiences with allografting in complete remission are encouraging, it is not as yet known if the relapse-free survival curves are indeed flat after 1–2 years.

Advances in transplantation therapy will depend on overcoming the current age limitations and tissue typing requirements, which limit the availability of this approach. Posttransplantation complications due to GVH disease and infection must be reduced but it should be realized, however, that a reduction in GVH disease may be associated with an increase in the rate of leukemic relapse. The immediate and perhaps long-term complications of the transplantation preparative procedure would be reduced if less intensive preparative regimens could be employed, a possibility raised by advances in assays predictive of drug responsiveness in leukemia (10,11).

CHEMOTHERAPY

Chemotherapy, as a means to treat patients in remission, has an advantage over transplantation in that it can be altered in time to suit the needs of the patient. One might also expect that chemotherapy would be less likely to produce long-term complications than does total-body irradiation and/or the administration of pharmacologic doses of alkylating agents. Whether this expectation is valid is unknown.

A major problem in comparing chemotherapy with allogeneic transplantation as "curative modalities" lies in the fact that there is no standard postremission chemotherapeutic regimen that can be compared to the relatively standardized transplantation regimens. The various studies of the efficacy of transplantation are relatively consistent in reporting a 30%–50% death rate during the first 1–2 years, with an apparent plateau after 2 years and little problem with leukemic relapse (4,5,12). In contrast, median remission duration and the percent long-term survivors among chemotherapy patients vary widely, ranging from short remissions with a few long-term survivors (13–15) to the data presented here. Which, if any, studies should be used to represent the overall efficacy of chemotherapy is unknown. Are the excellent results reported by Mayer et al (1) statistical anomalies or do they represent an advance in therapy? The Boston and Buffalo regimens differ from most other regimens in the intensity of consolidation therapy, and one would therefore like to believe that their superior results are truly reflective of the therapeutic approach and not merely chance observations. It is clear, however, that controlled trials comparing these intensive chemotherapeutic approaches with the more conventional chemotherapeutic programs are necessary.

A variety of "maintenance" and "consolidation" regimens are described in the literature. Some employ intensive therapy, while others are more "gentle." While the median durations of remission are reported, insufficient attention is paid to the percent of disease-free survivors at each year. Additionally, a comparison of the regimens per se is essentially impossible because of the large variety of chemotherapeutic agents used as well as the variety of schedules and routes of administration employed. One group of investigators may report the results of an "intensive consolidation" regimen, which in fact is less intensive than a "maintenance" regimen used by another group of investigators. To obviate this problem, consolidation or maintenance should be reported in terms of both the nadir and the duration of pancytopenia produced, the frequency at which the pancytopenia is produced, and the duration of therapy. Since different courses of therapy are administered and these produce different degrees of toxicity, perhaps the total time spent during each year at different levels of granulocytopenia and thrombocytopenia should be reported.

This approach to reporting treatment regimens would permit a comparison of different regimens in terms of intensity of therapy. The production of hematopoietic toxicity, however, is not synonymous with the production of an antileukemic effect, since there are many agents that are potent suppressors of normal hematopoiesis but have little or no antileukemic efficacy. Therefore, some effort should be made to rate treatment regimens in terms of their potential antileukemic efficacy. Perhaps chemotherapeutic agents employed in the regimens should be rated in terms of their antileukemic efficacy when administered as single agents. Agents that produce a complete remission rate of 20% could be class A drugs, those that produce 10%–20% complete remissions could be class B drugs, and those that produce < 10% complete remissions could be class C drugs. If the total doses of such rated agents administered (perhaps normalized to their optimum dose levels when used as single agents) were reported together with the frequency of administration, there would be some means for comparing the potential antileukemic efficacy of the different consolidation or maintenance regimens. Clearly, this approach is only a first approximation because of the complexities of drug interactions, dose-level effects, etc.

Recent studies at Roswell Park suggest that leukemic relapse is a complex event in that some patients relapse with drug-resistant disease while others relapse with leukemic cells that are sensitive to the drug with which they are being treated (16). While the former situation was expected, the latter was not. Taken together with the observation that plasma doxorubicin levels may become progressively lower in patients who are repeatedly treated with this agent (17), it is possible that on some occasions leukemic relapse occurs because of inadequate delivery of drug to the target cells in vivo. Leukemic relapse with drug-sensitive cells can also occur in the setting where patients cannot, for hematologic reasons, tolerate frequent or intensive courses of consolidation or maintenance therapy. Some of the latter patients should probably be considered for marrow transplantation once their inability to tolerate therapy is documented, while pharmacologic monitoring, with dosage escalation to insure the delivery of effective doses of chemotherapeutic agents during consolidation therapy, might prevent relapse of the former patients.

CONCLUSIONS

Both allogeneic marrow transplantation and intensive chemotherapy would be more effective if it were possible to tailor the treatment regimens to the characteristics of the individual patients. The data represented in figure 2A illustrate a typical dose-response curve for the sensitivity of leukemic cells to cytarabine, with exposure to increasing concentration of drug producing increasing cell death. Figure 2B demonstrates that the curve in figure 2A is really a mean population curve and does not represent the drug sensitivity curves of the individual patients. Substantial differences in the drug sensitivity of the leukemic cells of different patients suggest that the intensity and frequency of ablative therapy and/or consolidation therapy as well as the selection of the agents to be utilized should be determined by the sensitivity patterns of the leukemic cells, so that specific patients will not be overtreated or undertreated. Avoidance of the former would reduce the morbidity and lethality of therapy, while avoidance of the latter would reduce the incidence of leukemic relapse.

Little is known about the causes of late leukemic relapse. As noted above, it is difficult to imagine that any of the currently available chemotherapeutic agents are sufficiently selective in their effects to be able to kill the "last" leukemic cell while permitting a sufficient number of normal hematopoietic stem cells to survive. If this view is correct, then how can one account for long-term remissions and perhaps cures? Are there immunologic mechanisms capable of destroying or restraining the proliferation of leukemic cells? Is GVH disease a model of this phenomenon or are the possible effects of the graft-versus-leukemia reaction merely a manifestation of a GVH (in this case versus host hematopoietic tissue) reaction? Do chemotherapeutic agents restore the responsiveness of leukemic cells to mechanisms that normally regulate cell proliferation and differentiation? If these regulatory phenomena exist and play a role in the maintenance of long-term responses, are they manifestations of the effects of therapy on the leukemic cells or on the host? If there are normal host restraints, why does leukemia ever become clinically apparent? Do late recurrences actually represent the appearance of a second leukemia?

A comparison of allogeneic marrow transplantation during remission with intensive consolidation chemotherapy is also complicated by the fact that a chemotherapeutic regimen which produces a mortality rate (50% in the first year) comparable to that of the transplantation regimen has not as yet been administered nor is it likely to be. It appears to be more psychologically acceptable for this mortality rate to be produced as a result of transplantation than chemotherapy. While this acceptance is based at least in part on the curative potential of transplantation (18), it should be remembered that most modern chemotherapeutic regimens of even moderate intensity produce 10%–20% long-term survivors, suggesting that increases in the intensity of such therapy are indeed warranted. Unless or until chemotherapeutic regimens of comparable toxicity

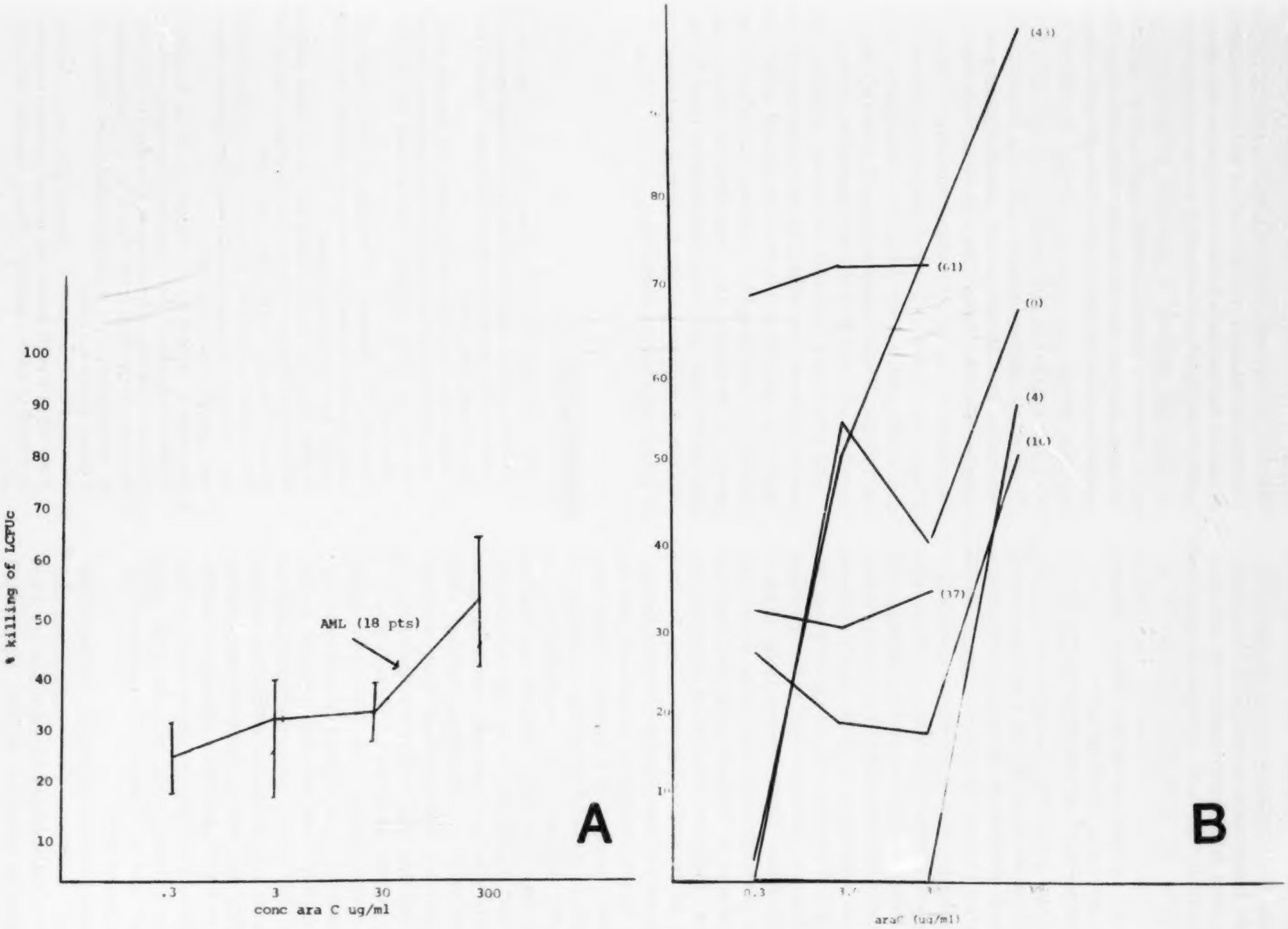

FIGURE 2.—Sensitivity of clonogenic leukemic cells (LCFUc) to cytarabine (ara C) in vitro. (A) Relationship between concentration of ara C and killing of LCFUc for 18 leukemic patients. Methods described in ref 6. (B) Relationship between concentration of ara C and killing of LCFUc from perspective of ara C sensitivity of LCFUc of individual patients. These data form part of pooled data represented in (A) above. Note that LCFUc of some patients are very sensitive to lowest ara C concentrations, while others are only killed by exposure to high ara C levels.

are administered to patients, a true comparison of the ability of the transplantation and chemotherapeutic approaches to produce long-term survival cannot be made. Furthermore, to the present time, no attempt has been made to compare the quality of life for patients who are treated with either of these two modalities. As each therapeutic approval becomes available for increasing numbers of patients, these comparisons will have to be made as well and put into perspective as the efficacy of each approach becomes more well-defined.

There is developing evidence that long-term remission can result even from a single course of remission induction therapy (19,20) and that second remissions are more likely to be induced in patients who relapse after therapy has been stopped than in patients who relapse while receiving chemotherapy. As already noted, second remissions, even in individuals who relapse while receiving therapy and who do not receive any consolidation or maintenance therapy, can be prolonged, with median durations in the range of 4–5 months (6). In this setting, intensive consolidation therapy might in fact produce long-term remissions. These observations suggest the possibility of an approach that takes advantage of the best features of chemotherapy and marrow transplantation. Patients who enter complete remission could be treated with only three or four courses of intensive consolidation chemotherapy. Patients who relapse would be reinduced into remission and then grafted if a suitable donor was available. While marrow allografting of patients in second remission appears to be associated with a higher relapse rate than when patients are grafted during their first remission, this may not be the case for patients who are in second remission after having relapsed while not receiving therapy, since these patients are less likely to have drug-resistant disease than patients who relapse while receiving chemotherapy. Older patients or those without a suitable donor who relapse could then be treated with more intensive or more prolonged chemotherapy than when they were initially treated. In this way a clinical trial could be carried out aimed at recognizing those individuals for whom the morbidity and mortality inherent in marrow transplantation or intensive prolonged chemotherapy is worthwhile. Hopefully, pretherapy assessment of the patient and his leukemic cells will reach the point that will permit such a decision to be made without conducting a clinical trial.

At the present time it is not known if allografting during remission and intensive consolidation chemotherapy are complementary therapies or are most suitable for the same subset of patients. Specialized facilities and physicians with specialized experience are necessary to effectively administer either of these therapeutic modalities, and when possible, patients should be referred to these specialized facilities for therapy. Allogeneic marrow grafting during clinical complete remission would seem to be the treatment of choice for patients in whom a cytogenetic abnormality that had been detected in the leukemic cells at diagnosis persists or increases in frequency despite intensive chemotherapy, since these patients are not likely to be cured by chemotherapy. The persistence and progression of cytogenetic abnormalities despite an apparent clinical remission appears to be particularly common in patients with Ph^1-positive acute leukemia (H. D. Preisler, M. Marinello, M. Brecher, et al, manuscript in preparation). Marrow transplantation may also be the treatment of choice for younger patients (< 20 years of age) who do not have ready access to centers that can safely administer intensive consolidation chemotherapy. These patients should probably be referred to an established transplantation center.

The treatment outcome for young patients who receive intensive consolidation chemotherapy or who receive an allogeneic marrow transplant in first remission is encouraging. Neither therapeutic approach is presently the treatment of choice, and both short-term and long-term studies must be carried out to define the role of each in the treatment of acute myelocytic leukemia.

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AMSA—A Promising New Agent in Refractory Acute Leukemia[1,2,3]

H. Jeffrey Lawrence,[4,5] Curt A. Ries,[6] Ralph D. Reynolds,[7] Jerry P. Lewis,[4,*] Maria M. Koretz,[8] and Frank M. Torti[8]

SUMMARY

Forty-five patients with various forms of acute leukemia refractory to usual methods of treatment were treated with AMSA at doses of 100–200 mg/m² iv daily for 5–7 days, for total doses of 500–1000 mg/m²/course. Among 41 evaluable patients, six (15%) achieved complete remissions, including two of 19 patients with typical acute nonlymphocytic leukemia, none of four with atypical acute nonlymphocytic leukemia, two of nine with chronic granulocytic leukemia in blast crisis, two of eight with acute lymphoblastic leukemia, and none of one with acute undifferentiated leukemia. Durations of complete remissions were 6, 7, 8, 12, 13, and 14 weeks. Four of the patients who died with infection during marrow hypoplasia 2–5 weeks after receiving AMSA had no evidence of leukemia on premortem bone marrow aspirates or postmortem examination. The primary toxic effects of AMSA were severe myelosuppression, stomatitis, and alopecia. One incident of life-threatening liver failure occurred. AMSA appears to be a promising agent for use in heavily pretreated patients with acute leukemia and chronic granulocytic leukemia in blast crisis.

[Cancer Treat Rep 66:1475–1478, 1982]

AMSA is an aminoacridine dicationic analog of the 4′-(9-acridinylamino) alkanesulfon anilides with a wide spectrum of antitumor activity in animal models (1). As one of several similar compounds, AMSA was chosen for study in human subjects primarily because of its superior activity in L1210 leukemia. (1).

The primary mechanism of the antitumor effect of AMSA is probably intercalation with DNA, a mechanism similar to that of anthracyclines (2). The drug is detoxified in the liver by conjugation with glutathione and excretion into the bile (3). Pharmacokinetic studies in humans with normal renal and hepatic function have shown that plasma disappearance of AMSA is biphasic, with a terminal-phase half-life of 7.4 hours (4). The half-life was mildly prolonged in a patient with severe renal failure (10.8 hours) and markedly prolonged in patients with severe hepatic dysfunction (17.5 hours).

Phase I studies have shown myelosuppression, stomatitis, and alopecia to be the most frequent toxic effects (5–8). Nausea, vomiting, and diarrhea have usually been mild. Rare cases of generalized convulsions and cardiac arrhythmias (some with sudden death) have been reported (9).

Responses in early clinical studies were primarily limited to patients with breast cancer, melanoma, and acute nonlymphocytic leukemia (ANLL) (8,10,11). The responses in ANLL included some complete remissions (CRs) (8), a finding that has fostered increasing interest in using AMSA for pa-

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[1] Received June 18, 1980; revised Feb 17, 1982; accepted Feb 25, 1982.

[2] Supported in part by Public Health Service grant CA-21744 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Presented in part at the 16th annual meeting of the American Society of Clinical Oncology, May 26–27, 1980.

[4] Division of Hematology and Oncology, Medical Center, University of California-Davis, Sacramento, CA.

[5] *Present address:* Division of Hematology and Medical Oncology, Oregon Health Sciences University, Portland.

[6] Cancer Research Institute, University of California, San Francisco.

[7] David Grant USAF Medical Center, Travis Air Force Base, CA.

[8] Northern California Oncology Group, Palo Alto.

[*] *Reprint requests to:* Jerry P. Lewis, MD, Division of Hematology and Oncology, Medical Center, University of California-Davis, Professional Bldg, 4301 X St, Sacramento, CA 95817.

tients with refractory acute leukemia. Preliminary studies have neither fully explored the range of AMSA activity in different types of leukemia nor clarified the optimal dose schedule. However, a report from the M. D. Anderson Hospital and Tumor Institute (Houston, TX) suggests that a total dose of approximately 525–630 mg/m²/course may be optimal (12).

The following report reviews the experience of the Northern California Oncology Group using three different doses of AMSA in a group of 45 heavily pretreated patients with several forms of acute leukemia.

MATERIALS AND METHODS

From June 1979 to December 1980, 45 patients with various forms of refractory acute leukemia were treated with AMSA. One additional patient was registered on the protocol but died before receiving his first dose. Four patients (three with ANLL and one with chronic granulocytic leukemia in blast crisis [CGL-BC]) were considered inevaluable for response, three because of major protocol violations and one because of incomplete data. The general characteristics of the treated group and the subtypes of leukemia are shown in table 1.

Patients were eligible for this phase II study if they had ANLL, smoldering leukemia, acute undifferentiated leukemia (AUL), acute lymphoblastic leukemia (ALL), or CGL-BC. All patients with ANLL had received at least two courses of cytarabine and anthracyclines. Patients with ALL and AUL had received several courses of vincristine and prednisone as well as anthracyclines. All patients with CGL-BC had received at least one prior course of chemotherapy consisting of hydroxyurea, vincristine, prednisone, 6-mercaptopurine, and anthracyclines in various combinations. All patients signed informed consent papers.

TABLE 1.—General patient characteristics

Characteristic	No. of patients
Total	45
Age (yrs)	
Mean: 36.1	
Range: 9–69	
Sex	
Male	27
Female	18
Histologic type	
Typical ANLL	22
Acute granulocytic (FAB M1 and M2)	17
Acute promyelocytic (FAB M3)	1
Acute myelomonocytic (FAB M4)	4
Atypical ANLL	4
ANLL arising from preleukemia	2
Smoldering ANLL	2
CGL-BC	10
ALL	8
AUL	1
Karnofsky performance status (%)	
10–50	12
60–100	32
Not given	1

Physical examinations and blood cell counts were performed daily during induction therapy and at least weekly following induction until maximum response was determined. Bone marrow aspirations were obtained prior to initiation of treatment and every 1–2 weeks after induction therapy was completed until the maximum response was determined. For patients in CR, blood cell counts were obtained every 3 weeks and bone marrow aspirations every 6–9 weeks.

CR was defined as $< 5\%$ blast infiltration of the marrow, a peripheral granulocyte count $> 2000/\text{mm}^3$, a platelet count $> 100{,}000/\text{mm}^3$, and no symptoms related to leukemia. Partial remission (PR) was defined by any combination of (*a*) a 5%–25% blast infiltration of the marrow, (*b*) a granulocyte count of 1000–2000/mm³, (*c*) a platelet count of 50,000–100,000/mm³, and (*d*) 1%–5% circulating blasts.

AMSA was given in three different dose schedules: 100 mg/m² daily × 5 days, 200 mg/m² daily × 5 days, and 120 mg/m² daily × 7 days, with dose reductions for pre-existing liver dysfunction. The drug was supplied by the National Cancer Institute, Bethesda, MD, in vials containing 75 mg of AMSA. The contents of each vial were mixed with 13.5 ml of a lactic acid diluent provided with the drug. The mixed solution of AMSA and lactic acid was diluted in a minimum of 250 ml of 5% dextrose in water and infused through an iv line over 30–60 minutes (approximately 2–3 mg/m²/minute). Most patients were hospitalized during the induction therapy and throughout the subsequent period of hypoplasia. Patients entering remission were given AMSA maintenance therapy at a dose of 120 mg/m² every 3 weeks on an outpatient basis.

RESULTS

Response

A prompt antileukemic effect was observed in nearly every patient. In marrow aspirates obtained 1 week after completion of therapy, hypoplasia was universally observed, with marrow cellularities of $\leq 20\%$. The overall response rate for 41 evaluable patients was 29% (six CRs and six PRs). Responses were distributed among histologic types as shown

TABLE 2.—Best overall response for evaluable patients by type of leukemia

Response	No. of patients				
	ANLL	ALL	CGL-BC	AUL	Total
CR	2	2	2	0	5
PR	5	1	0	0	7
No response	11	2	2	1	16
Early death	5	3	5	0	13
Total	23	8	9	1	41

in table 2. CRs were achieved in two of 19 patients (11%) with typical ANLL, none of four with atypical ANLL (two cases of smoldering leukemia and two cases of ANLL arising from a clear-cut myelodysplastic syndrome), two of eight (25%) with ALL, and two of nine (22%) with CGL-BC. CRs were observed in none of five patients who received cycles of < 600 mg/m^2 total dose of AMSA, in four of 27 (15%) given 600–850 mg/m^2, and two of nine (22%) treated with > 850 mg/m^2. Thirteen patients died during marrow hypoplasia within 4 weeks of treatment (early deaths), and four had no evidence of leukemia on premortem marrow aspiration or autopsy examination.

The two CRs in patients with typical ANLL were achieved with total doses of 800 and 840 mg/m^2 and lasted through 13 and 14 weeks of maintenance therapy. The two CRs in patients with ALL were accomplished with doses of 840 and 1000 mg/m^2 and lasted 6 and 8 weeks. The patient with an 8-week CR had T-cell ALL which relapsed after maintenance therapy was withheld because of unusual toxic effects (*see* below). Two patients with CGL-BC achieved CRs after receiving 840 and 1000 mg/m^2, with return to stable phase of CGL and normalization of peripheral counts. One patient refused all maintenance therapy and relapsed in 12 weeks; the other relapsed in 7 weeks. All CRs were achieved after one cycle of therapy. Five patients received two cycles each without response. Of note were two patients with prior CNS involvement who developed CNS relapses within 1 month of AMSA therapy. Median survival was 9 weeks for the entire group, 28 weeks for complete responders, 21 weeks for partial responders, and 10 weeks for nonresponders, excluding those dying during hypoplasia in the first 4 weeks. This difference in survival times by response levels was statistically significant ($P < 0.001$). There was no significant difference ($P = 0.13$) in survival by histology (ANLL, ALL, and CGL-BC).

Toxicity

Side effects were substantial. All patients developed severe myelosuppression, with marrow recovery occurring in 3–5 weeks. Every patient required transfusions of rbcs and/or platelets. One patient died 4 weeks after his second course of AMSA, with sepsis and persistent marrow hypoplasia.

Severe stomatitis was noted in 18 patients, including one case of life-threatening severity. The frequency of severe stomatitis was dose-related. Severe stomatitis occurred in no courses of < 600 mg/m^2, 12 of 36 courses of 600–850 mg/m^2, and six of ten courses of > 850 mg/m^2. The stomatitis was characterized by painful ulceration and patchy necrosis of the oropharyngeal mucosa with a predilection in some cases for the lateral margins of the tongue. Three instances of moderately severe diarrhea were observed. Nausea and vomiting were usually mild, with only four cases of severe emesis.

Hyperbilirubinemia (serum bilirubin > 1.0 mg/dl) was noted in 23 cycles of therapy, with a range of serum bilirubin levels of 1.1–25.6 mg/dl (median, 2.5). Four patients had bilirubin elevations > 10 mg/dl. Levels of serum alkaline phosphatase of greater than twice normal (100 IU/liter) were noted in 16 cases, but in no patient exceeded 500 IU/liter.

In the patient with T-cell ALL, the SGOT increased rapidly to 675 U/liter 4 weeks after a course of 1000 mg/m^2 of AMSA. One week later, when the SGOT had decreased to 480 U/liter, the patient received two daily doses of 100 mg/m^2 of AMSA as maintenance therapy. The third day he presented with jaundice and a serum bilirubin of 6.6 mg/dl. Surface antigen for hepatitis B virus was not detectable. Jaundice, hyperbilirubinuria, and marked transaminasemia persisted for 2 weeks, when percutaneous liver biopsy revealed severe centrilobular necrosis and fatty degeneration. AMSA was withheld but the patient developed progressive hepatic dysfunction, with a maximum serum bilirubin of 21.7 mg/dl, a maximum SGOT of 885 U/liter, and markedly prolonged prothrombin time. Three months after receiving his first course of AMSA, the patient relapsed and died.

Other toxic effects included marked alopecia (all patients), mild to moderate phlebitis (two), and possible mild hearing loss (one). One case of congestive heart failure was observed, but no serious arrhythmias were noted. No significant neurologic or renal toxic effects were seen.

DISCUSSION

This study demonstrates that AMSA can produce CRs in heavily pretreated patients with ANLL, ALL, or CGL-BC. The response rate in patients with ANLL is comparable to that obtained with 5-azacitidine, an agent commonly used in refractory ANLL but having very little activity in ALL (13). Moreover, AMSA showed activity in two particularly refractory types of leukemia, ie, T-cell ALL and CGL-BC.

The response rate of ANLL in this study was less than the 30% CR rate reported in the M. D. Anderson series (12). However, eight of their 11 CRs were in patients with only one prior treatment regimen. All but five of our patients had received two or more courses of chemotherapy before entry in this study. Moreover, unlike the M. D. Anderson series, we observed CRs in ALL.

The optimal dose and schedule of AMSA are uncertain. One group of investigators had previously reported CRs with total doses of approximately 500 mg/m^2 (8); however, we observed no responses at this level. We therefore increased the dose using two different schedules (200 mg/m^2/day × 5 days and 120 mg/m^2/day × 7 days). The toxic effects of these relatively high-dose regimens, particularly stomatitis, were substantial and at the highest dose tested (1000 mg/m^2) were unacceptable. Thus our experience suggests that the optimal dose may be approximately 800 mg/m^2 given over 5–7 days. Even at this "intermediate" dose, myelosuppression is severe and often prolonged (3–5 weeks), with most patients requiring intensive support for many days after treatment.

Severe liver dysfunction, as seen in the patient with T-cell ALL, has not been previously reported with AMSA, perhaps because other studies have used lower doses of the drug. Preclinical studies in dogs, however, have shown significant hepatotoxicity (14). The evidence in this case is circumstantial but suggests at least a contributive role for AMSA in the hepatic failure of this patient. It is also pertinent that many of our other patients developed transient elevations of serum bilirubin and alkaline phosphatase.

In our study, iv AMSA did not confer protection against CNS relapse. This observation confirms the findings of pharmacokinetic studies that only very low spinal fluid concentrations of the drug can be obtained from iv administration (4).

The role of AMSA in maintenance therapy is unclear, although the short duration of remission in this study suggests little benefit from the drug given once every 3 weeks. Nevertheless, AMSA is now being tested at several centers in combination with other agents such as 5-azacitidine and cytarabine in an effort to produce higher response rates in refractory leukemia. Preliminary results are promising (15,16).

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Clinical Evaluation of Succinylated *Acinetobacter* Glutaminase-Asparaginase in Adult Leukemia[1,2]

Raymond P. Warrell, Jr,* Zalmen A. Arlin, Timothy S. Gee, Ting-Chao Chou, Joseph Roberts, and Charles W. Young[3,4]

SUMMARY

We treated 13 adult patients with acute leukemia or chronic myelocytic leukemia (CML) in blast phase using succinylated *Acinetobacter* glutaminase-asparaginase (SAGA) administered on a daily dose schedule. SAGA reduced the peripheral blast count in two patients with acute lymphoblastic leukemia and two with blastic CML; however, no patient achieved either complete or partial remission. Marked central nervous system toxic effects (encephalopathy and coma) were observed, limiting treatment in patients whose disease appeared responsive; this effect finally prompted early discontinuance of the trial. Other toxic effects observed included nausea, hyperglycemia, and respiratory alkalosis. Hypersensitivity reactions to the enzyme were not seen. Pharmacologic analyses showed that prolonged blood glutamine depletion was achieved only by daily enzyme administration; however, we noted the importance of performing amino acid analysis on blood which was deproteinized immediately following phlebotomy. Our results demonstrate excessive central nervous system toxicity when glutaminase-asparaginase is administered on a daily schedule. Because of this effect, we propose that future trials of similar enzymes be limited to short courses of enzyme therapy, possibly with the addition of antimetabolites or amino acid analogs, which could enhance the antitumor effect without increasing toxicity.

[Cancer Treat Rep 66:1479–1485, 1982]

Enzymatic depletion of "nonessential" amino acids exploits an important biologic difference between normal and neoplastic cells, namely, the inability of some tumor cells to synthesize certain amino acids when deprived of exogenous supplies. The first such enzyme introduced clinically, *Escherichia coli* asparaginase, has substantial activity as a single agent in acute lymphoblastic leukemia (ALL) (1). Furthermore, Rausen et al (2) have shown that the addition of asparaginase to standard chemotherapy markedly increases the complete remission rate achieved in relapsed patients, thus confirming the earlier preclinical observations of Burchenal (3). Moreover, Capizzi (4) demonstrated that, depending upon the timing of enzyme administration, asparaginase can either enhance or negate the therapeutic effects of methotrexate. Several trials have since reported the usefulness of sequential methotrexate-asparaginase chemotherapy for patients with refractory leukemia (5,6).

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[1] Received May 12, 1981; revised Feb 8, 1982; accepted Mar 4, 1982.

[2] Supported in part by National Research Service Award CA-09207 (*R. P. Warrell, Jr.*), and by Public Health Service grants CA-08748, CA-15860, CA-05826, and CA-18856 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Developmental Chemotherapy (*R. P. Warrell, Jr.*, and *C. W. Young*) and Hematology/Lymphoma (*Z. A. Arlin* and *T. S. Gee*) Services and the Laboratories of Pharmacology (*T.-C. Chou*) and Experimental Enzyme Therapy (*J. Roberts*), Memorial Sloan-Kettering Cancer Center, New York, NY.

[4] We thank Mr. Alvin Donner and Mrs. Clara Gordon for technical assistance and Ms. Shelley Hantman for assistance with preparation of the manuscript.

**Reprint requests to:* Raymond P. Warrell, Jr, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

The clinical use of asparaginase is limited by its toxicity and by its lack of significant antitumor activity in other disorders (2,7). Also, the development of hypersensitivity and the rapid induction of acquired resistance to the enzyme represent additional problems for patients whose disease may initially be susceptible (8). The glutaminase-asparaginase enzyme isolated from *Acinetobacter* has a much broader spectrum of antitumor activity in vitro than *E. coli* asparaginase (9,10); furthermore, tumor cell lines initially sensitive to this enzyme do not acquire resistance after retreatment (11). While the native enzyme is too rapidly cleared from plasma to be of clinical utility, Holcenberg et al (12) showed that succinylation of the enzyme molecule markedly prolongs the plasma half-life without affecting biologic potency (12).

Our initial clinical evaluation of succinylated *Acinetobacter* glutaminase-asparaginase (SAGA) showed that bolus iv doses only transiently lowered blood glutamine to nondetectable levels (< 5 μM), with rapid recovery thereafter owing to influx of glutamine from extravascular sources. Several doses administered daily were required to achieve prolonged blood glutamine depletion (13). The dose-limiting toxic effect of this schedule was central nervous system (CNS) depression. This effect, however, was dose-related and completely reversible. Moreover, it appeared that potentially severe CNS reactions could be avoided by withholding therapy if asterixis became clinically evident. A daily dose of 320 IU/m^2 was tolerated by most patients and produced some evidence of antileukemic effect. This report summarizes our subsequent experience with this enzyme in the treatment of adult patients with leukemia.

PATIENTS AND METHODS

Patients

Adults with acute leukemia or chronic myelocytic leukemia (CML) in blast crisis were eligible for entry into this study after they failed standard treatment for their disease. All patients had recovered from the toxic effects of previous chemotherapy and had adequate hepatic and renal functions (serum bilirubin and creatinine $\leq$ 1.5 mg/100 ml). Pretreatment evaluation included a bone marrow aspirate, cbc and platelet count, screening biochemical profile, urinalysis, electrocardiogram, and chest roentgenogram. Patients were monitored with the following laboratory tests: cbc and platelet count, daily; serum electrolytes, BUN, and glucose, every other day; and screening biochemical profile and serum amylase, weekly. Serial coagulation tests (prothrombin time, partial thromboplastin time, thrombin time, reptilase time, fibrinogen, fibrin degradation products, and factor VIII coagulant activity) were performed every 3 days. On selected patients, serial determinations of plasma glutaminase activity and whole-blood concentrations of glutamine, glutamate, and ammonia were made according to methods described previously (13). Blood samples used for the latter determinations were deproteinized immediately after phlebotomy by the addition of cold perchloric acid to whole blood (final perchloric acid concentration, 10%). Signed informed consent was obtained in advance and the study was approved by the Clinical Investigations Committee of the Memorial Sloan-Kettering Cancer Center.

Treatment Protocol

Each patient had a skin test by the intradermal injection of 0.1 IU of enzyme/kg of body weight. If neither erythema nor urticaria appeared after 1 hour of observation, the prescribed dose of SAGA was infused iv over 15–20 minutes. All treatment was administered on an inpatient basis.

Therapy was initiated at a daily dose of 300 IU/m^2; treatment was withheld if mental confusion or asterixis developed and was resumed when these signs disappeared. In the absence of CNS toxicity, the dose was escalated by 100 IU/m^2/day every 2–3 days to tolerance, until some evidence of antileukemic activity (eg, fall in peripheral blast count) was noted. Patients were considered to be adequately treated if marrow hypocellularity was achieved or if CNS toxicity appeared without objective response of disease. CNS toxicity was graded as follows: 1+, lethargy; 2+, disorientation with asterixis; and 3+, coma. Responses were assessed as previously described (14).

Enzyme Preparation

The enzyme was prepared according to procedures developed by Roberts et al (9) and was kindly supplied by Kyowa Hakko Kogyo Co, Tokyo, Japan. The enzyme was succinylated as previously described (12,13) and lyophilized. Prior to administration, the enzyme was dissolved in normal saline solution and sterilized by ultrafiltration. Enzyme used in the clinical study had a specific L-glutaminase activity of 200 IU/mg of protein.

RESULTS

Biochemical Determinations

As we noted previously (13), single doses of SAGA did not deplete immediately deproteinized whole

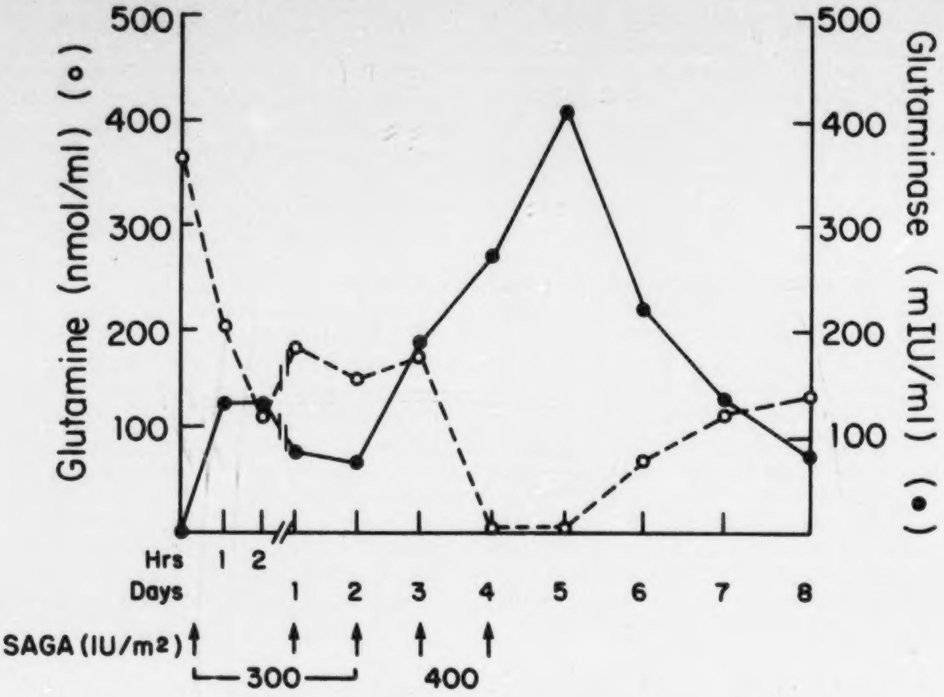

FIGURE 1.—Effect of daily dose administration of SAGA on whole-blood concentrations of glutamine (○) and plasma levels of glutaminase activity (●).

blood of its glutamine content. Several doses administered daily were required before glutamine concentrations fell to consistently undetectable levels (< 5 μM) (fig 1). During SAGA treatment, concentrations of blood glutamate continued to rise, indicating ongoing hydrolysis of glutamine entering from extravascular sources. Ammonia levels rose initially and then appeared to reach a plateau concentration (fig 2).

Clinical Responses

Eleven adult patients with leukemia were entered in this study; two additional patients who received SAGA during the phase I trial at constant daily doses of 320 and 500 IU/m², respectively, are also included in this analysis. Patient diagnoses and clinical responses are presented in table 1.

No patient achieved complete or partial remission. SAGA reduced circulating blast cells in two patients with ALL and in two with CML in blast phase; however, the leukemic cells from both CML patients expressed a lymphoid phenotype (ie, lymphoblastic morphology with elevated terminal deoxynucleotidyl transferase activity) (15). No patient with acute nonlymphoblastic leukemia showed evidence of an antileukemic effect.

One of the patients with blast phase CML achieved a reduction in bone marrow blast cells from > 90% to 20%; no marrow effects were noted in other patients. We also noted a dose-response relationship in this patient (fig 3). Whereas daily administration of SAGA at doses ≤ 400 IU/m² produced no antileukemic effect, dose escalation to ≥ 500 IU/m² caused a pronounced reduction of circulating blast cells which persisted for several weeks after therapy was discontinued.

Toxicity

As expected, CNS toxicity was dose-limiting. Contrary to our expectations from the phase I study, this effect proved clinically unmanageable and prompted early discontinuation of the study. Of the 13 patients treated, five became comatose; three additional patients developed a marked encephalopathy. These CNS effects cleared in all patients within 72 hours of halting enzyme therapy. One patient experienced a fatal intracranial hemorrhage as a result of trauma sustained while he was encepha-

TABLE 1.—Clinical effects of SAGA in patients with leukemia

Patient No.	Diagnosis*	Duration of therapy (days)	Total dose (IU/m^2)	CNS toxicity	Wbc response†
1	CML-Bl	13	6300	3+	↓
2	AMMoL	5	2600	3+	↑
3	ALL	5	1700	3+	0
4	AML	3	1500	3+	0
5	ALL	4	1300	3+	↓
6	AML	4	1300	2+	0
7	AML	4	1300	2+	0
8	ALL	10	4200	1+	0
9	ALL	10	3200	1+	↓
10	CML-Bl	10	3200	1+	↓
11	AMMoL	7	2100	1+	↑
12	ALL	5	1700	1+	0
13	CML-Bl	3	900	2+	↑

*CML-Bl = chronic myelocytic leukemia in blast phase; AMMoL = acute myelogenous, myelocytic leukemia; and AML = acute myelogenous leukemia.

†Wbc = total peripheral blood wbc count. An increase (↑) is ≥ 30% increment over initial value; a decrease (↓) is ≥ 1 log reduction from initial value. Intermediate changes are recorded as 0.

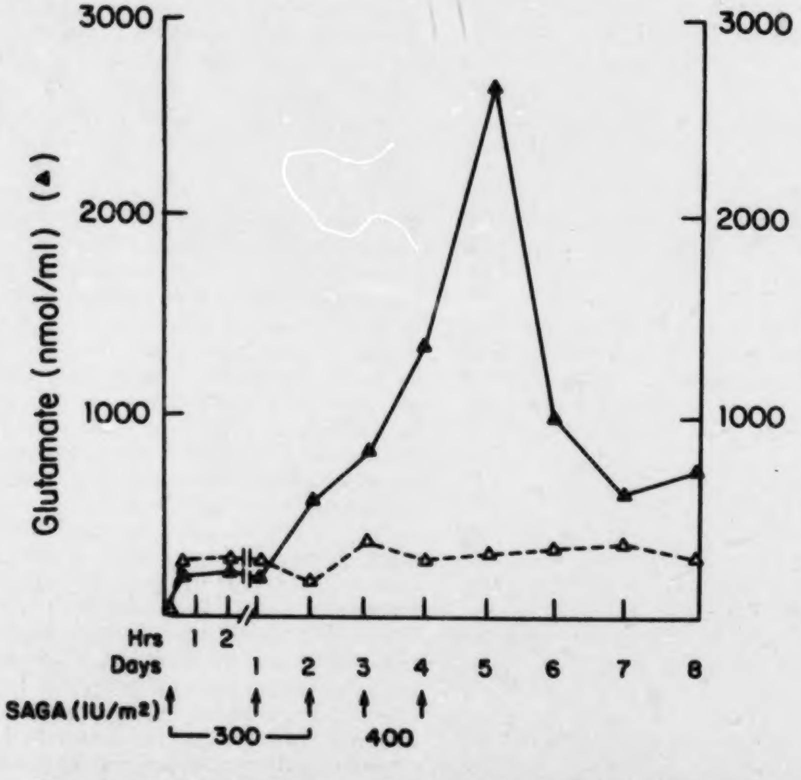

FIGURE 2.—Whole-blood concentrations of ammonia (△) and glutamate (▲) following daily dose administration of SAGA.

lopathic; this death was considered to be drug-related.

Respiratory alkalosis from hyperventilation, probably induced by the elevation in blood ammonia, was noted in all patients. The patient described in figure 3 experienced a gradual decline in serum bicarbonate concentration to 10 mEq/liter; on Day 18 he developed acute, severe hyperglycemia which produced coma, renal failure, and disseminated intravascular coagulation from which he eventually recovered. It is likely that the severity and abrupt onset of this disorder was secondary to exhaustion of buffering capacity stemming from renal bicarbonate loss as compensation for the primary respiratory alkalosis. No disorders of the blood coagulation system were observed in other patients.

Other toxic effects included nausea and vomiting, which consistently occurred at doses ≥ 500 IU/m². No effect on serum levels of calcium or albumin was noted; no patient developed clinical pancreatitis or hyperamylasemia. Contrary to a previous clinical report of a similar enzyme (16), none of our patients experienced hypersensitivity reactions to this enzyme.

DISCUSSION

SAGA produced excessive CNS toxicity when administered on the dose schedule described. The incidence of substantially greater toxicity in this trial at a dose lower than that administered in a pediatric trial (17) suggests that adults may be more susceptible to these CNS effects. As in hepatic encephalopathy, these toxic effects may be caused by sustained elevations of ammonia concentration in blood and cerebrospinal fluid (13,18), which result from the enzymatic deamidization of both glutamine and asparagine. Because of this toxicity, we cannot draw confident conclusions regarding the theoretic advantages of SAGA relative to *E. coli* asparaginase (ie, broader activity and lack of induced resistance).

Although only patients with lymphoblastic phenotype showed clinical response, therapy with higher doses of enzyme for more prolonged duration might produce responses in patients with acute nonlymphoblastic leukemia, provided that amelioration of the CNS effects could be achieved. Such responses have been reported in clinical trials with similar glutaminase-asparaginase enzymes (19).

It is conceivable that an alternative dose schedule might increase the therapeutic index for this drug. Daily administration was selected in view of the high incidence of hypersensitivity reactions seen with intermittent enzyme dosage (2,20) and because of pharmacologic data obtained during our phase I evaluation. Previous studies have reported quite

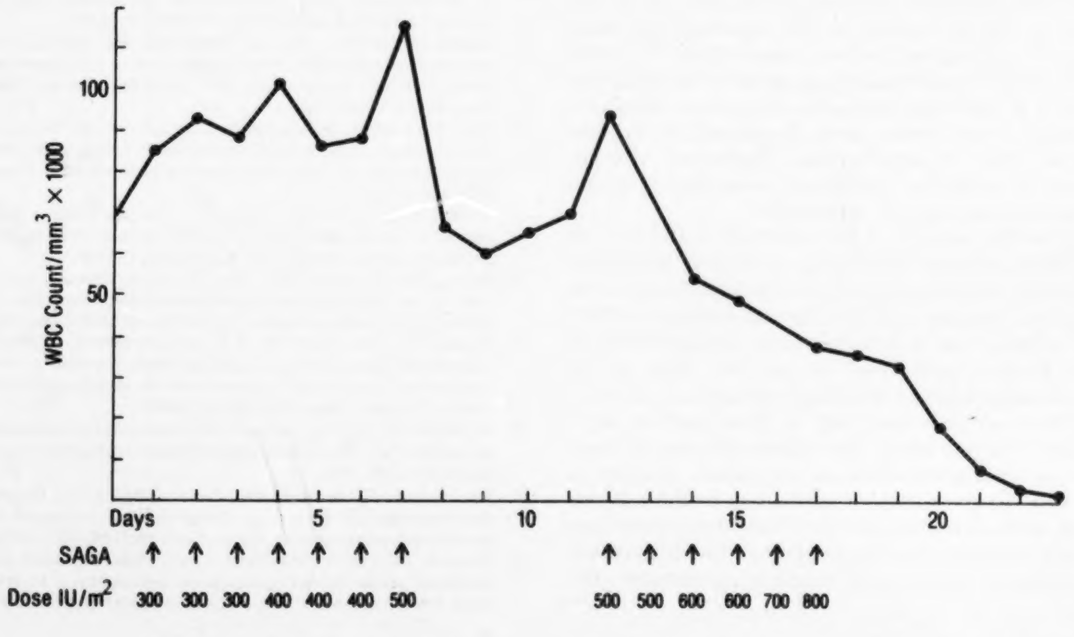

FIGURE 3.—Dose-response relationship between increasing doses of SAGA and peripheral wbc count in a patient with blast phase CML.

prolonged depletion of plasma glutamine (for several days) following single doses of glutaminase-asparaginase (19,21). We found, however, that single doses of SAGA only transiently lowered blood glutamine to nondetectable levels when the analysis was performed on blood in which the enzyme was inactivated by deproteinization at the bedside (13). This method measures total amino acid concentration in whole blood (ie, that contained in both the plasma and intracellular compartments). However, the failure to achieve prolonged glutamine depletion in whole blood following enzyme administration cannot be ascribed to the persistence of an intraerythrocytic "glutamine reservoir." Using this method, we have shown that whole-blood glutamine levels *are* depleted by single doses of enzyme; however, these levels rapidly recover (13). We again noted that serial daily doses of SAGA were required to achieve and maintain continuous glutamine depletion in whole blood (fig 1). Since the enzyme appears to be confined to intravascular volume (13), it seems likely that low levels of circulating glutaminase in blood which is not immediately deproteinized will hydrolyze glutamine ex vivo, whereas actual in vivo blood glutamine concentrations are higher as a result of continuous glutamine influx from extravascular sources. It is not clear, however, what level of blood (or tissue) glutamine depletion is necessary to realize antitumor effects. While we noted a definite dose-response relation to SAGA in one of our patients (fig 3), Tallal et al (22) reported that daily doses of asparaginase which ranged from 10 to 5000 IU/kg all produced equivalent rates of complete remission in childhood leukemia. Moreover, blood asparagine levels were rarely measured during the clinical trials of asparaginase. Therefore, the necessity of achieving prolonged depletion of amino acids in blood remains uncertain.

Given the toxicity of daily administration, an alternative schedule employing short daily courses of enzyme in combination with an antimetabolite or an amino acid analog might be of greater clinical utility. The combination of the glutamine analogs DON (6-diazo-5-oxo-L-norleucine) or acivicin with glutaminase-asparaginase achieves substantially greater antitumor effects than any of these agents alone (23,24). Furthermore, the concurrent use of these analogs with glutaminase-asparaginase appears to prevent the formation of antibodies to the enzyme in experimental animals (24). Thus, the clinical use of such combinations might achieve the desired antineoplastic effects while minimizing enzyme toxicity.

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The Course of Nausea and Vomiting After High-Dose Cyclophosphamide[1,2]

John H. Fetting,[3,4,*] Louise B. Grochow,[3] Marshal F. Folstein,[4] David S. Ettinger,[3] and Michael Colvin[3,5]

SUMMARY

We studied the course of nausea and vomiting after high-dose cyclophosphamide (50–75 mg/kg) in 25 patients. Nausea was assessed with a visual analog scale and vomiting by patient report. The reliability and validity of these methods of assessment were established in separate studies of oncology patients. Nausea and vomiting developed between 6 and 12 hours after the start of a 1-hour cyclophosphamide infusion in about two thirds of the patients. Peak symptoms were at 12 hours. Vomiting had subsided in most of the patients by 24 hours, but nausea persisted. Although there was significant individual variation in the course of nausea and vomiting, we could identify no demographic, disease, drug, or treatment factors which were associated with more significant nausea and vomiting. Some of the variability in the onset, severity, and duration of nausea and vomiting may be related to individual differences in the metabolism of and susceptibility to cyclophosphamide. The visual analog scale may provide information about nausea not obtained by the more frequently employed categoric verbal rating scales. Suggestions for the treatment of nausea and vomiting after high-dose cyclophosphamide are made on the basis of the findings.

[Cancer Treat Rep 66:1487–1493, 1982]

Cyclophosphamide is a frequently prescribed antineoplastic drug. Large doses (≥ 50 mg/kg) have been employed in the treatment of small cell carcinoma of the lung (1) and multiple myeloma (2) and in preparation for bone marrow transplantation (3–5). Unfortunately, such large doses cause severe vomiting (6–8). However, there are no careful descriptions in the literature of the course of nausea and vomiting after cyclophosphamide treatment. Such a description may improve our understanding of the toxicology of cyclophosphamide emesis and suggest better antiemetic treatment plans. We report on the nausea and vomiting caused by high-dose cyclophosphamide in 25 patients who were studied by reliable and valid methods for the measurement of nausea and vomiting.

METHODS

Sample

Table 1 provides data that describe our study group. Twenty-five hospitalized patients (20 males and five females) with a mean age of 42 years (range,

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[1] Received Sept 10, 1981; revised Feb 18, 1982; accepted Mar 4, 1982.

[2] Supported in part by Public Health Service grants CA-17970 and CA-17448 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Fetting was a 1980–1981 recipient of an American Cancer Society Regular Clinical Fellowship.

[3] The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

[4] Department of Psychiatry, The Johns Hopkins Hospital, Baltimore, MD.

[5] We appreciate the comments of A. H. Owens, Jr, MD, and we thank Mimi Yacoub and Nancy B. Kramer for their excellent secretarial assistance.

[*] *Reprint requests to:* Dr. John H. Fetting, The Johns Hopkins Oncology Center, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—Characteristics of 25 patients who received high-dose cyclophosphamide

Patient No.	Age (yrs)	Sex	Disease*	Cyclophosphamide dose (mg/kg)	Antiemetics	Allopurinol	Emesis from previous chemotherapy
1	47	M	HCL	75	Prochlorperazine	Yes	No
2	21	M	AA-BMT	50	Prochlorperazine	No	No
3	64	M	MM	60	Prochlorperazine	Yes	Yes
4	40	M	CML	75	Antihistamines	Yes	No
5	19	M	ANLL	75	Fluphenazine, chlorpromazine	Yes	Yes
6	19	F	ALL	75	Prochlorperazine	No	No
7	20	M	ALL-BMT	50	Prochlorperazine	Yes	Yes
8	55	F	SCCL	75	Prochlorperazine	No	No
9	45	M	AA-BMT	50	Chlorpromazine	No	No
10	21	M	ALL	75	Prochlorperazine	Yes	Yes
11	63	M	MM	60	Prochlorperazine	Yes	Yes
12	31	M	HCL-BMT	50	Antihistamines	Yes	No
13	71	M	MM	60	Prochlorperazine	Yes	Yes
14	33	M	HCL-BMT	50	Antihistamines	Yes	Yes
15	52	M	ANLL	75	Prochlorperazine	Yes	Yes
16	62	M	SCCL	75	Prochlorperazine	Yes	No
17	48	F	AA-BMT	50	Prochlorperazine	No	No
18	27	M	AA-BMT	50	Prochlorperazine	No	No
19	54	M	CML	75	Antihistamines, prochlorperazine	Yes	No
20	24	M	AA-BMT	50	Phenobarbital	No	Yes
21	52	M	SCCL	75	Prochlorperazine	No	No
22	51	M	SCCL	75	Prochlorperazine	Yes	No
23	59	F	MM	60	Chlorpromazine, prochlorperazine	Yes	No
24	42	M	ANLL	75	Antihistamines	Yes	Yes
25	32	F	MM	50	Chlorpromazine	Yes	Yes
N = 25	Mean, 42.1	20 M 5 F		Mean, 63.6		Yes = 17; No = 8	Yes = 11; No = 14

*AA = aplastic anemia, ALL = acute lymphocytic leukemia, ANLL = acute nonlymphocytic leukemia, CML = chronic myelogenous leukemia, HCL = hairy cell leukemia, MM = multiple myeloma, SCCL = small cell carcinoma of the lung, and BMT = bone marrow transplant.

19–71) participated in the study. Cyclophosphamide (50–75 mg/kg) was given as a 1-hour iv infusion to 21 patients, as a 1–2-hour infusion to three patients, and as a 4-hour infusion to one patient. Nine patients received 50 mg/kg, four received 60 mg/kg, and 12 received 75 mg/kg. The mean dose of cyclophosphamide was 63.6 mg/kg. Patients received no other chemotherapeutic agent in the week before or 24 hours after the administration of cyclophosphamide. Cyclophosphamide was administered for small cell carcinoma of the lung (four patients), multiple myeloma (five), acute leukemia (five), chronic leukemia (two), and in preparation for bone marrow transplantation for a variety of diseases (nine). The patients were ambulatory and able to care for themselves (Eastern Cooperative Oncology Group performance status 0 or 1). They were ineligible for the study if they had any condition or were receiving any medication which regularly causes vomiting. Eleven patients had received emesis-producing chemotherapy previously, and of these, four had received high-dose cyclophosphamide. Various antiemetic therapies were employed. Fifteen patients received prochlorperazine alone, one received prochlorperazine combined with antihistamine antiemetics, two were treated with chlorpromazine alone,

one received chlorpromazine plus fluphenazine, four were treated with antihistamines, and one was treated with phenobarbital. Twelve of the 15 patients who received prochlorperazine alone were part of a pilot study to see if ≥ 12 hours of pretreatment with 6-hourly doses of 5–10 mg of prochlorperazine was a promising antiemetic approach. The remaining 13 patients had their antiemetic treatment initiated at the time their chemotherapy was given or when they developed nausea and vomiting. No patients received cannabinoids as antiemetics. Seventeen patients were receiving allopurinol at the time they received cyclophosphamide. No other medication was prescribed with significant frequency. Patients were allowed food, fluids, and activity ad libitum after receiving cyclophosphamide.

Nausea and Vomiting Assessment

Nausea and vomiting were assessed by self-report. The first assessment was completed just prior to the cyclophosphamide infusion and then every 3 hours for 24 hours. Nausea was evaluated using a visual analog scale bounded on one end by "not at all nauseous" and at the other end by "extremely nauseous." Patients indicated the nausea they were experiencing at the time they completed the scale by making a slash mark at an appropriate place on the 100-mm line. The nausea score was the number of millimeters from the "not at all nauseous" end of the line to the patient's mark. At each assessment, patients also reported the number of times they had vomited in the preceding 3-hour period. Vomiting was defined as the expulsion of gastric contents.

Although the reliability and validity of the analog method are established for a variety of conditions (9), these studies were repeated for nausea in separate studies of oncology patients. Reliability of the visual analog scale as a method for measuring nausea was assessed by test-retest. Patients were asked to mark the line and then were asked to mark it again 10–15 minutes later. Eighteen patients (11 males and seven females; mean age, 43 years) provided 27 pairs of nausea test-retest scores over a wide range of nausea scores for the reliability study.

The validity of the analog method for measuring nausea was established by comparison of the patient's nausea score with the assessment of a single clinical observer (J. H. Fetting) who scored the patient as nauseous or not after a brief clinical interview. The observer was unaware of the patient's self-report nausea score.

We established a cut-off score which indicated nausea. A random sample of 26 oncology patients (14 males and 12 females; mean age, 46 years) who stated they were not nauseous were asked to mark the analog scale. The mean score in this group was 10, with a standard deviation of 12. A score of 22 (one standard deviation above the mean) was then arbitrarily set as representing a "case" of nausea by self-report.

Twenty randomly selected oncology patients (13 males and seven females; mean age, 41 years) comprised the sample for the validation trial. Two-by-two tables were used to examine the relationship between self-report scores above and below 22 and the assessment of the clinical observer.

The self-report of vomiting was also validated by comparison with the report of a blinded clinical observer. The same sample of 20 patients used for the nausea validation trial was employed. A two-by-two table was employed to compare the patient's self-report of vomiting with that of the clinical observer.

RESULTS

Reliability and Validity of Self-Report Assessment

The coefficient of correlation between the 27 test-retest nausea scores was 0.83. This was judged to be an acceptable level of reliability. In the validity trial, a self-report score of 22 was used for comparison with the report of the clinical observer. A two-by-two table was employed to analyze the relationship between self-report nausea scores and the report of the clinical observer. With a score of ≥ 22 indicating nausea, there was a significant relationship between the patient report and that of the clinical observer ($P < 0.025$ by chi-square test). The relationship between self-report of vomiting and the report of the observer was significant at the $P < 0.001$ level by chi-square test.

Nausea and Vomiting After High-Dose Cyclophosphamide

Twenty-three of 25 (92%) patients experienced nausea (analog scale score ≥ 22) at some time during the 24 hours after cyclophosphamide. Twenty-three (92%) patients vomited, including one of the two patients who was not nauseous at any 3-hour assessment. One patient had nausea and one episode of vomiting just prior to the cyclophosphamide infusion. He attributed that to 5 mg of iv prochlorperazine. This medication was discontinued and his nausea and vomiting subsided over the next 6 hours. Both nausea and vomiting increased dramatically between 6 and 9 hours after cyclophosphamide, presumably in response to cyclophosphamide.

Figure 1 shows the number of patients developing nausea over time. Twenty-two of 23 (96%) patients developed nausea within 12 hours of drug administration, 15 of 23 (65%) first developed nausea between 6 and 12 hours after cyclophosphamide, and seven of 23 (30%) did so within the first 6 hours. The patient who first reported nausea at 18 hours had reported vomiting at several previous assessments, suggesting that his nausea may have been intermittent. Figure 2 shows the mean nausea scores of the sample at each assessment time; it also demonstrates that nausea increases dramatically between 6 and 12 hours after cyclophosphamide. Although the peak nausea score was at 12 hours, there was no statistically significant difference (by t test) among the scores from 9 to 21 hours. Thirteen of 25 (52%) patients reported their peak nausea score at 12 hours. The next most frequent peak nausea time was at 15 hours at 16% of the patients. Fifty-four percent of the patients were still nauseous 24 hours after cyclophosphamide.

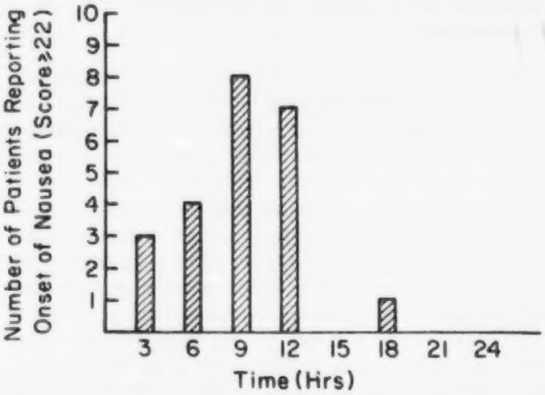

FIGURE 1.—Development of nausea over time after high-dose cyclophosphamide. Cyclophosphamide was infused over the 1st hr in 84% of the patients.

Figure 3 shows the development of vomiting in our patients over time. Vomiting tended to have a somewhat later onset than nausea. Sixteen of 23 (70%) patients developed vomiting between 6 and 12 hours after cyclophosphamide but only three of 23 (13%) developed vomiting in the first 6 hours. Four of 23 (17%) patients developed vomiting > 12 hours after cyclophosphamide. Figure 4 shows the mean number of vomiting episodes during the previous 3 hours reported at each assessment time. Vomiting increased dramatically between 6 and 12 hours after cyclophosphamide. Peak vomiting was reported at 12 hours but there was no statistically significant difference (by t test) between the number of vomiting episodes reported at 12 hours and those reported at 9, 15, and 18 hours. Vomiting had significantly declined from the 12-hour peak at 21 hours, and by 24 hours the mean value was less than one episode. Only 33% of the patients reported vomiting between 21 and 24 hours after cyclophosphamide. Patients reported vomiting an average of 8.6 times during the 24 hours after cyclophosphamide.

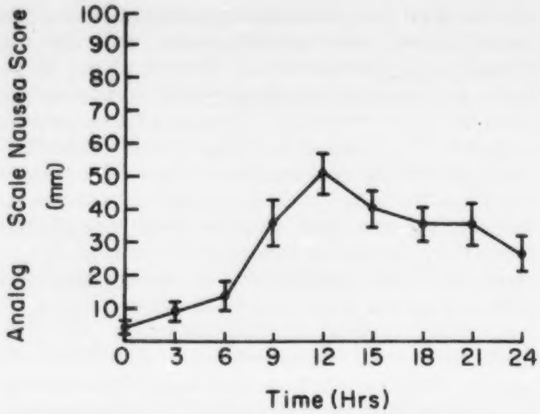

FIGURE 2.—Mean nausea scores of patients (± SE) over time after high-dose cyclophosphamide.

As can be seen from the preceding data, there is significant individual variation in the onset, sever-

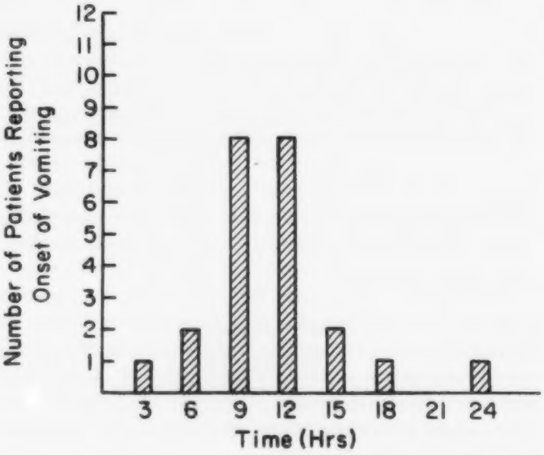

FIGURE 3.—Development of vomiting over time after high-dose cyclophosphamide. Cyclophosphamide was infused over the 1st hr in 84% of the patients.

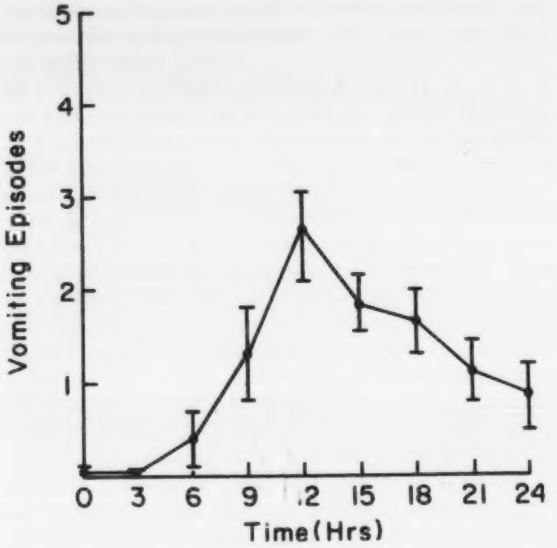

FIGURE 4.—Mean No. of vomiting episodes (± SE), reported by patients during the previous 3 hrs, over time after high-dose cyclophosphamide. Cyclophosphamide was infused over the 1st hr in 84% of the patients.

ity, and duration of nausea and vomiting after high-dose cyclophosphamide.

We attempted to identify factors in subsets of our patients which were associated with more severe nausea and vomiting. We subdivided our patients according to age (above and below the mean age of 42 years), sex, disease, and cyclophosphamide dose (50 and 60 vs 75 mg/kg). In addition, we contrasted patients who had been treated with and without allopurinol and prochlorperazine pretreatment. Finally, we compared patients who had previously received emesis-producing chemotherapy with those who had not. For each of these subgroups we compared mean values (by t test) for onset, duration, and severity of nausea and vomiting. Dividing our sample in these ways did not reveal any subgroups with a significantly different course of nausea and vomiting after high-dose cyclophosphamide.

DISCUSSION

We found that nausea and vomiting occurred very predictably after high-dose cyclophosphamide. There was a 6–12-hour latency period from drug administration until the development of nausea and vomiting in 65%–70% of our patients. Peak symptoms occurred 12 hours after cyclophosphamide but the entire period from 9 to 18 hours was marked by severe nausea and vomiting. Although vomiting had decreased significantly by 24 hours after cyclophosphamide, most of the patients were still nauseous. Since 84% of our patients received cyclophosphamide as a 1-hour infusion, we were unable to examine the relationship between rate of cyclophosphamide administration and the course of nausea and vomiting. A 6-hour delay in the onset of nausea and vomiting after cyclophosphamide has been commented on previously. It was suggested that this delay was related to the time required for the hepatic metabolism of the drug to cytotoxic and presumably emetic metabolites (10).

We could not demonstrate a dose-response relationship between cyclophosphamide dose and nausea and vomiting over the range of 50–75 mg/kg. Although this may be related to small patient size, it is also possible that at such large doses nausea and vomiting are at maximal levels. If this were the case, nausea and vomiting after more modest doses of cyclophosphamide might demonstrate a more typical dose-response relationship. An inverse relationship has been noted between mechlorethamine dose and vomiting severity and latency in cats (11).

We were unable to identify other factors which contributed to the individual variation we observed in the course of nausea and vomiting. There was no apparent relationship between age, sex, or underlying disease and the course of nausea and vomiting. Since the number of female patients and the number of patients with each underlying disease were small, it is possible that true differences could have been missed. We could not demonstrate a systematic effect of allopurinol on nausea and vomiting. Allopurinol competes with cyclophosphamide for hepatic microsomal enzymes but has not been found to appreciably alter cyclophosphamide's cytotoxic effects (12). Since 92% of our patients received phenothiazine antiemetics, it is impossible to discern an effect of this class of drugs on the course of nausea and vomiting. On the other hand, this high percent receiving phenothiazines provided for antiemetic homogeneity in our patients. It would be more difficult to analyze the course of nausea and vomiting after cyclophosphamide in patients receiving many different antiemetics. Since phenothiazines are supposedly effective antiemetics for postchemotherapy vomiting (13), the duration and severity of nausea and vomiting observed after cyclophosphamide may underestimate the true values. Since nausea and vomiting remained severe in these patients despite phenothiazine antiemetics, we believe antiemetic treatment had a relatively minor impact on the time course of nausea and vomiting. The use of more effective antiemetics, however, might change the time course as well as the severity of nausea and vomiting after high-dose cyclophosphamide. Although there are reports (14) that patients who have previously received emesis-producing chemotherapy might have a different course of nausea and vomiting than naive patients, we could not demonstrate it in these patients. Although not systematically studied, we asked 20 of our patients to choose from a list of 13 common hospital activities those which palliated and exacerbated their nausea and vomiting. Seventy percent indicated that sleeping palliated nausea and vomiting, 5% felt it exacerbated the syndrome, and 25% had no preference. Eating and drinking exacerbated nausea and vomiting for 55% and palliated for none. Since we did not attempt to control or systematically assess such variables, it is unclear how they may have influenced our results. Control of such variables as oral intake has been attempted in some antiemetic studies (15) but apparently not in others (16,17). Other investigators have written about the effect of activities of daily living on the course of nausea and vomiting after chemotherapy (18). Finally, it is not known what effect individual differences in the metabolism of cyclophosphamide might have on the course of nausea and vomiting.

We employed a reliable, valid, visual analog scale to measure nausea. By deriving a cut-off score for nausea we were also able to divide our patients categorically according to the presence or absence of nausea. The categoric analysis was more useful for determining the onset and duration of nausea, whereas the analog scale was most helpful for following the severity of nausea over time, especially in an individual patient. The visual analog scale has rarely been employed in the measurement of nausea associated with chemotherapy or in antiemetic studies (19). Most such studies use a categoric verbal rating scale (15–17). The visual analog has been widely used in the assessment of pain, mood, appetite, etc (9,20–22). There are reports that the visual analog scale is more sensitive than a categoric scale for measuring pain (23,24). It seems possible that the visual analog scale could contribute increased sensitivity to the measurement of nausea as well. Self-report is an inexact method for monitoring emesis. Since vomiting is an observable behavior involving many physiologic changes, some direct measurement system could be devised for monitoring it. Preliminary studies of respiratory inductance plethysmography for monitoring cat vomiting suggest that this method may be applicable to the study of emesis in man (25).

Based on this study we can suggest some modifications in the antiemetic strategies for high-dose cyclophosphamide. Antiemetics are usually given on a fixed-dose schedule. Since the nausea and vomiting associated with high-dose cyclophosphamide varied in intensity over the 24-hour observation period, a fixed-dose antiemetic schedule may not be optimal. It may be worthwhile to increase the antiemetic dose or dose interval to cover the peak in nausea and vomiting which occurs 12 hours after the initiation of the cyclophosphamide infusion. Since more aggressive antiemetic treatment at this time may cause significant sedation, it may be useful to start high-dose cyclophosphamide infusions in the afternoon so that peak symptoms and antiemetic-induced sedation occur during usual sleeping hours. Since there may be an increased risk of aspiration pneumonia with this approach, appropriate precautions should be taken. Additionally, the sedated patient must be awakened every 1–2 hours to urinate if hemorrhagic cystitis is to be avoided.

Antiemetic therapy for many anticancer drugs is only partially effective. Careful descriptions of the course of emesis after these agents will improve our understanding of their toxicology and allow us to devise more specific antiemetic strategies.

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Essential Thrombocythemia: Response During First Year of Therapy With Melphalan and Radioactive Phosphorus: A Polycythemia Vera Study Group Report[1,2]

Scott Murphy,* David S. Rosenthal, Aleksander Weinfeld, Jean Briere, Guy B. Faguet, William H. Knospe, Stephen A. Landaw, John Laszlo, Anthony V. Pisciotta, Anthony P. Tartaglia, Judith D. Goldberg, Paul D. Berk, Paul B. Donovan, and Louis R. Wasserman[2]

SUMMARY

Thirty-one patients with essential thrombocythemia were randomized to receive either melphalan or radioactive phosphorus as myelosuppressive therapy. Twenty-seven patients were evaluable for response. Of 13 patients treated with melphalan, 11 had a complete response (platelet count $< 450{,}000/\text{mm}^3$) at 3 and 6 months. This response rate was significantly better than the response to radioactive phosphorus. The response rates were similar at 12 months. No significant toxicity was observed with either regimen.

[Cancer Treat Rep 66:1495–1500, 1982]

Thrombocytosis is a frequent manifestation of all the myeloproliferative disorders (MPD) (1). There are reports of patients who tolerate strikingly high platelet levels for many years without clinical sequelae (2). However, several reports (3–6) involving small numbers of patients suggest that such patients are at increased risk for thrombotic and hemorrhagic complications and that such complications can be reduced in frequency and severity by lowering the platelet count with myelosuppressive therapy. Within the large spectrum of MPD, there is a subgroup of patients who have thrombocytosis without accompanying manifestations of polycythemia vera, chronic granulocytic leukemia, or myelofibrosis (agnogenic myeloid metaplasia). This subgroup has been labeled essential or primary thrombocythemia (ET). Again, despite reports of small series, there are no prospective studies of a large group of ET patients which would define their response to myelosuppressive therapy. This report describes the experience of the Polycythemia Vera Study Group (PVSG) during the first year of treatment of ET with melphalan (Alkeran) and radioactive phosphorus (^{32}P).

MATERIALS AND METHODS

Between 1975 and 1980, the PVSG conducted two studies, PVSG 04 and PVSG 10, for the registration and study of patients with thrombocytosis secondary to MPD (7). Both protocols were identical except that in the second study, PVSG 10, patients < 40 years of age were excluded and the dose of ^{32}P was increased (*see* below). Patients were randomized in blocks of four within each country of origin to receive either melphalan or ^{32}P. The initial dose of melphalan was 10 mg/day for 5 successive days. The dose was then reduced to 2 mg/day for the following 3 weeks. The subsequent dose of melphalan was adjusted for continuous maintenance of the platelet count in the range of 200,000–400,000/mm^3. In PVSG 04, the initial dose of ^{32}P was 2.3 millicuries/m^2 iv, not to exceed 5.0 millicuries/dose. During the course of this protocol, it was recognized that this dose of ^{32}P was not consistently effective

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[1] Received Nov 9, 1981; revised Mar 3, 1982; accepted Mar 17, 1982.

[2] Supported by Public Health Service grant CA-10728 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. *See* Appendix for a list of participating investigators.

**Reprint requests to:* Scott Murphy, MD, Department of Medicine, Cardeza Foundation for Hematologic Research, Thomas Jefferson University, 1015 Walnut St, Philadelphia, PA 19107.

in producing a prompt reduction in the platelet count. Therefore, in PVSG 10, the initial dose was increased to 2.9 millicuries/m^2, not to exceed 5 millicuries/dose. ^{32}P was administered at intervals of 3 months, if needed, to maintain the platelet count < 450,000/mm^3. Response to therapy was evaluated at 3, 6, and 12 months by recording the lowest platelet count in three time intervals after start of therapy: 9–17, 20–32, and 44–60 weeks. The patient was listed as having a complete response (CR) if that platelet count was < 450,000/mm^3. If that platelet count was in the range of 450,000–600,000/mm^3, 600,000–1,000,000/mm^3, or > 1,000,000/mm^3, the patient was listed as having a good partial response (PR), a poor PR, or no response, respectively. Crossover to the alternate therapy arm was permitted at 6 months if there was no response to the primary treatment.

Among patients entered and randomized over a 5-year period, 31 were subsequently identified who met the following strict criteria for the diagnosis of ET: (*a*) Platelet count ≥ 1,000,000/µl on at least two separate determinations. (*b*) Absence of the Philadelphia chromosome in metaphases obtained from marrow aspirates. (*c*) Normal rbc mass measured with ^{51}chromium-labeled rbcs (8) (this requirement was waived if the hemoglobin was < 13 g/100 ml; four patients). (*d*) Iron present on iron stain of marrow (if absent, a patient was considered eligible if a trial of oral iron therapy for 1 month resulted in no increase in hemoglobin; three patients). (*e*) No fibrosis on marrow biopsy (if fibrosis was present, a patient was considered eligible if the fibrosis was less than one third of the marrow sectional area and was not associated with splenomegaly and leukoerythroblastic blood picture; one patient). (*f*) Thrombocytosis could not be attributed to some other identifiable cause, ie, infection, chronic inflammatory disease, malignancy, previous splenectomy, etc.

Within each study, characteristics of patients on the two treatment regimens were compared using methods of descriptive statistics. Similarly, response rates in the two treatments were compared, adjusting for protocol using the Mantel-Haenszel

TABLE 1.—Initial characteristics of patients by protocol and treatment group

	No. of patients				
	PVSG 04		PVSG 10		
Characteristic	^{32}P	Melphalan	^{32}P	Melphalan	Total
No. in group	8	7	9	7	31
Age (yrs) at entry					
< 50	1	2	2	0	5
50–70	4	2	5	4	15
> 70	3	3	2	3	11
Sex					
Male	5	4	3	5	17
Female	3	3	6	2	14
Spleen					
Enlarged	3	2	2	2	9
Not enlarged	5	5	7	5	22
Liver					
Enlarged	2	0	1	0	3
Not enlarged	6	7	8	7	28
Platelet count (cells × 10^3/mm^3)					
< 1000	0	1	0	0	1
1000–1499	6	4	5	5	20
1500–2000	1	1	3	1	6
> 2000	1	1	1	1	4
Wbc count (cells × 10^3/mm^3)					
4.5–12	5	4	4	4	17
> 12	3	3	5	3	14
Hgb (g/100 ml)					
9–11.9	3	2	2	2	9
12–15	4	4	6	2	16
> 15	1	1	1	3	6

TABLE 2.—Response to therapy by treatment and protocol (evaluable patients)

	No. of patients (%)			
	PVSG 04		PVSG 10	
	^{32}P	Melphalan	^{32}P	Melphalan
3-mo response				
CR	1 (17)	4 (67)	5 (63)	7 (100)
Good PR	1 (17)	1 (17)	1 (13)	0 (0)
Poor PR	1 (17)	1 (17)	2 (25)	0 (0)
No response	3 (50)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)
Totals	6 (100)	6 (100)	8 (100)	7 (100)
6-mo response				
CR	1 (17)	4 (67)	5 (63)	7 (100)
Good PR	2 (33)	2 (33)	1 (13)	0 (0)
Poor PR	2 (33)	0 (0)	1 (13)	0 (0)
No response	1 (17)	0 (0)	1 (13)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)
Totals	6 (100)	6 (100)	8 (100)	7 (100)
12-mo response				
CR	3* (50)	3 (50)	5 (63)	5 (71)
Good PR	0 (0)	2 (33)	3 (37)	2 (29)
Poor PR	3 (50)	0 (0)	0 (0)	0 (0)
No response	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	1 (17)	0 (0)	0 (0)
Totals	6 (100)	6 (100)	8 (100)	7 (100)

*1 patient had CR at 12 mos after switching to melphalan at 6 mos. He had had no response to ^{32}P at 6 mos.

chi-square test. Four of the 31 patients identified with ET were ineligible for the evaluation of therapy for the following reasons: one PVSG 04 patient randomized to receive melphalan was lost to followup, and three patients randomized to ^{32}P (two in PVSG 04 and one in PVSG 10) were treated with only 1.5–1.7 millicuries/m^2, less than the prescribed initial dose. Of the 27 patients eligible for evaluation, 12 were randomized in PVSG 04, six in each treatment group. Of the 15 eligible patients randomized in PVSG 10, seven were treated with melphalan and eight with ^{32}P.

Characteristics of these ET patients at time of randomization to each study (PVSG 04 and PVSG 10) are shown by treatment group in table 1. In general, within each study, patients in the two treatment groups are comparable. Fifty-five percent of these patients were male; 84% were > 50 years old. No patients < 50 years of age were randomized to melphalan in PVSG 10. Twenty-nine percent of the ET patients had a palpable spleen, but the largest recorded was only 4 cm below the costal margin. Ten percent had a palpable liver which was judged clinically to be enlarged. Mild anemia and leukocytosis were characteristic of a minority of patients. Twenty-nine percent had peripheral hemoglobin < 12 g/100 ml, but none had hemoglobin < 9 g/100 ml. The peripheral wbc count was > 12,000/mm^3 in 45%. The initial platelet count was < 1,000,000/mm^3 in one PVSG 04 patient treated with melphalan; two subsequent measurements, however, were > 1,000,000/mm^3. Of the 31 patients, seven had a history of thrombosis, seven had a history of hemorrhage, two had a history of both, and 15 had a history of neither. Thrombotic manifestations included angina (four patients), venous thrombosis (three), transient ischemic attacks (three), cerebrovascular accident (one), and peripheral arterial disease (one). Hemorrhagic manifestations included epistaxis (five patients), easy bruising (three), postoperative hemorrhage (one), gastrointestinal hemorrhage (one), and retinal hemorrhage (one).

RESULTS

The CR rate at 3 months for PVSG 04 was 17% for ^{32}P compared with 67% for melphalan (table 2). Similarly, for PVSG 10 at 3 months the CR rate of 100% for melphalan was somewhat higher than the 63% CR rate for ^{32}P. In fact, the CR rate is significantly higher for melphalan than for ^{32}P, adjusting for protocol at 3 months (Mantel-Haenszel $\chi^2_1 = 4.0$, corrected for continuity, $P = 0.05$). The CR rates at 6 months similarly favor melphalan. However, at 12 months, the CR rates for the two treatments are comparable, adjusting for protocol.

All patients treated with melphalan achieved a CR at one of the three evaluation intervals during the 12 months; in fact, all patients except two in PVSG 04 reached CR at 3 months. Only four of the six PVSG 04 patients receiving ^{32}P ever achieved a CR and three[3] of these four patients required 12 months to do so. In PVSG 10, all eight patients treated with ^{32}P achieved a CR at some point. Five of the eight did so within 3 months. Thus, in PVSG 10, the higher ^{32}P dose appeared to be associated with more rapid and complete response.

The immediate toxicity of these two regimens was negligible; no significant leukopenia or thrombocytopenia was reported. Two patients had disease complications during the study period. One patient had a cerebrovascular accident 9 days after the initiation of melphalan therapy when the platelet count was 730,000/mm^3; one patient had a deep vein thrombosis after 6 months of therapy with ^{32}P when the platelet count was 1,350,000/mm^3. There were no hemorrhagic complications.

DISCUSSION

The patients reported on in this study represent the initial experience of the PVSG with therapy for ET. In general, the patient characteristics are consistent with descriptions of small groups of patients in the literature. Splenomegaly, hepatomegaly, mild anemia, and leukocytosis were observed, but only in a minority of patients. Only one third of our patients had platelet counts > 1,500,000/µl and only one half had symptoms of thrombosis or hemorrhage. Many of the small series in the literature described patients with more striking increases of the platelet count and more striking symptoms. It is likely that this reflected the tendency to report dramatic examples of a relatively rare disease. We know of no bias within our institutions which would lead to the exclusion of more severely affected cases.

All patients were treated with myelosuppressive therapy, with a goal of permanently lowering the platelet count to < 450,000/mm^3. This was easily and safely accomplished using melphalan, confirming the report of Bensinger et al (6) in a smaller series. The response rate to ^{32}P was significantly slower. The CR rate at 3 months was less than that observed with melphalan, and six of the 14 patients treated with ^{32}P had a platelet count > 600,000/mm^3 at that point. This result may be partially explained by dose since the results were somewhat better when the ^{32}P dose was raised from 2.3 to 2.9 millicuries/m^2 in PVSG 10. Either drug was tolerated during the study period without significant toxicity.

These studies do not address two important questions. The first is whether patients without symptoms of thrombosis or hemorrhage need to be treated. The second is whether patients who have achieved a good remission with a course of therapy need to continue in a maintenance program which maintains a normal platelet count. Our results show that remission and maintenance can be accomplished easily. However, both of the agents employed will almost certainly increase the risk of second malignancies, particularly if they are employed in a maintenance program (9,10). The data presented here are limited to experience with remission induction and the first year of management. The patients continue under observation; their long-term followup will be recorded in a future report.

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[3] Includes one patient in PVSG 04 treated initially with ^{32}P who had a 12-month CR after switching to melphalan at 6 months. He had had no response to ^{32}P at 6 months.

APPENDIX

The collaboration of the members of the Polycythemia Vera Study Group who are listed below is acknowledged. Without their contributions to the study, the results mentioned in all papers would not have been possible.

L. R. Wasserman, MD, *chairman*	
W. R. Arrowsmith, MD	Ochsner Foundation Clinic
*S. P. Balcerzak, MD	Ohio State University Hospitals
R. Berger, MD	Institut de Recherches Sur Les Leucemie-Paris
P. D. Berk, MD	Mount Sinai Medical Center
N. I. Berlin, MD	Northwestern University Medical Center
E. C. Besa, MD	Medical College of Pennsylvania
M. H. Block, MD	University of Colorado Medical Center
*J. Briere, MD	Centre Hospitalier Regional de Brest-France
L. H. Brubaker, MD	Medical College of Georgia
E. W. Campbell, MD	Medical College of Ohio

M. Coleman, MD	New York Hospital-Cornell Medical Center
M. R. Cooper, MD	Bowman Gray School of Medicine
*A. A. Cooperberg, MD	Jewish General Hospital-Montreal
*P. B. Donovan, MD	Mount Sinai Medical Center
C. Dresch, MD	Institut de Recherches Sur Les Leucemie-Paris
J. T. Ellis, MD	New York Hospital-Cornell Medical Center
*G. B. Faguet, MD	Medical College of Georgia
F. H. Gardner, MD	University of Texas Medical Branch (Galveston)
J. D. Goldberg, ScD	Mount Sinai Medical Center
R. D. Goldman, MD	University of Oregon School of Medicine
*K. Goldstein, MD	George Washington University Medical Center
G. L. Holcomb, MD	Mayo Clinic
P. Jacobs, MD	University of Cape-Town-South Africa
M. E. Kaplan, MD	Minneapolis V. A. Medical Center
*D. B. Kimball, MD	Walter Reed Army Medical Center
*W. H. Knospe, MD	Rush Presbyterian-St. Luke's Medical Center
E. H. Kraut, MD	Ohio State University Hospitals
*S. A. Landaw, MD	Syracuse V. A. Medical Center
*J. Laszlo, MD	Duke University Medical Center
J. H. Lawrence, MD	University of California-Donner Laboratories
V. Loeb, Jr, MD	Washington University Medical Center
R. O. Lundy, MD	Brooke Army Medical Center
*O. R. McIntyre, MD	Dartmouth Medical College
L. M. Meyer, MD	Brooklyn-St. Albans V. A. Medical Center
B. Modan, MD	Chaim Sheba Medical Center-Israel
*S. Murphy, MD	Thomas Jefferson University Medical College
Y. Najean, MD	Institut de Recherches Sur Les Leucemie-Paris
W. D. Noyes, MD	University of Florida Medical Center (Gainesville)
B. S. Pasternack, PhD	New York University Medical Center
*M. C. Perry, MD	University of Missouri Medical Center
P. Peterson, MD	New York Hospital-Cornell Medical Center
R. M. Petitt, MD	Mayo Clinic
R. V. Pierre, MD	Mayo Clinic
*A. V. Pisciotta, MD	Wisconsin Medical College
J. Poindexter, MD	Medical College of Virginia
K. Rai, MD	Long Island Jewish-Hillside Medical Center
H. Rapapport, MD	City of Hope Medical Center
D. S. Rosenthal, MD	Peter Bent Brigham Hospital
Y. Sacks, MD	University of Oregon School of Medicine
A. Sawitsky, MD	Long Island Jewish-Hillside Medical Center
R. F. Schilling, MD	University of Wisconsin Medical Center
E. B. Silberstein, MD	University of Cincinnati Medical Center
R. T. Silver, MD	New York Hospital-Cornell Medical Center
*M. N. Silverstein, MD	Mayo Clinic
*C. L. Spurr, MD	Bowman Gray School of Medicine
H. L. Stauffer, MD	University of California-Donner Laboratories
*A. P. Tartaglia, MD	Albany Medical Center
L. Tatarsky, MD	Rambam Government Hospital-Israel
R. O. Wallerstein, MD	University of California-San Francisco
*J. K. Weick, MD	Cleveland Clinic Foundation
*A. Weinfeld, MD	University of Göteborg-Sweden
N. J. Weinreb, MD	Mount Sinai Medical Center
J. Whang-Peng, MD	National Institutes of Health
R. Wittman, MD	Mount Sinai Medical Center
D. Wurster-Hill, PhD	Dartmouth Medical College

*Indicates patient contribution to this study.

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Management of Pelvic Complications of Malignant Urothelial Tumors With Combined Intra-Arterial and Iv Chemotherapy[1]

Christopher J. Logothetis,* Melvin L. Samuels, Sidney Wallace, Vincent Chuang, Antonio Trindade, Cynthia Grant, Thomas P. Haynie, III, and Douglas E. Johnson[2]

SUMMARY

Twenty-nine patients with local-regional pelvic recurrences of malignant urothelial tumors were treated with combined intra-arterial and iv chemotherapy. 5-FU (1 g/m^2) was infused daily for 5 days via bilateral percutaneously placed hypogastric catheters. Doxorubicin (25 mg/m^2) was infused iv on Days 1 and 2 and mitomycin (5 mg/m^2) was infused iv on Days 3 and 4 of each course. Seventeen patients (58%) demonstrated an objective decrease in the size of the pelvic mass. All four patients with one of the adenocarcinoma variants of malignant urothelial tumors responded to therapy, while the three patients with squamous transformation of a transitional cell carcinoma failed to respond. The median survival of the responding patients was significantly longer (52 weeks) than that of nonresponding patients (28 weeks) (P = 0.002). Patients with initial low-volume pelvic disease demonstrated durable control of local residual tumors, with four of six patients having negative repeat cystoscopic examinations.

[Cancer Treat Rep 66:1501–1507, 1982]

The treatment of patients with locally advanced bladder cancer is unsatisfactory. Many modalities have been introduced for the palliation of pelvic symptoms, with only modest success (1). Attempts with intra-arterial chemotherapy infused via bilateral hypogastric artery catheters have had encouraging results. 5-FU and mitomycin have each been extensively studied and have achieved consistent objective response (2,3).

The advantage of intra-arterial over iv chemotherapy can be theoretically attributed to the higher concentration of drug delivered to the infused site. The drug concentration achieved in the target site is inversely proportional to the flow rate of the infused vessel and directly proportional to the total quantity of drug administered. Systemic toxicity is determined by the rate of drug elimination, while local toxicity is determined by the drug concentration in the tissue (4). The hypogastric vessels offer a readily approachable vascular system with a relatively low flow rate. 5-FU is an ideal agent for arterial infusion because of the rapid drug elimination and modest bone marrow toxicity when it is delivered as a continuous infusion (5,6).

In an attempt to increase the rate and duration of previously described objective responses in the management of pelvic recurrences of malignant urothelial tumors, we have adopted 5-FU as the single agent to be administered intra-arterially at maximum tolerated dosages. To this we have added the simultaneous iv infusion of doxorubicin and mitomycin. These two agents were selected because of their previously described activity against malignant urothelial tumors (7,8) and their clinically documented synergism (9).

The 29 patients treated were selected because each had as a dominant clinical problem the pres-

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[1] Received Nov 13, 1981; revised Mar 5, 1982; accepted Mar 19, 1982.

[2] Departments of Medicine (*C. J. Logothetis, M. L. Samuels, A. Trindade, C. Grant,* and *T. P. Haynie, III*), Diagnostic Radiology (*S. Wallace* and *V. Chuang*), and Urology (*D. E. Johnson*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Christopher J. Logothetis, MD, Department of Medicine, Rm W-725, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

ence of threatening local pelvic complications of the tumor. To distribute the drug more accurately in each catheter, we employed radionuclide angiography. We believe this technique reflects more faithfully the distribution of the infused drug and allows for the estimation of the proportional infusion of each catheter to the tumor bed.

MATERIALS AND METHODS

Twenty-nine patients with predominant pelvic complications of malignant urothelial tumors were treated with a combination of intra-arterial and systemic chemotherapy.

Patients were included in the study if the predominant clinical complication of the tumor was local-regional pelvic disease. Patients were excluded only if they had pre-existing myocardial disease precluding the use of doxorubicin. Twenty-five patients had tumor arising in the bladder, one in the ureter, two in the prostatic ducts, and one in the urachus. All patients were initially evaluated with a complete history and physical examination and metastatic surveys consisting of chest X-rays, bone scan, liver-spleen scan, and iv pyelogram. Abdominal computed axial tomography, ultrasound, and lymphangiography were often employed. Baseline cardiac scans with ejection fractions were performed. Initial blood tests included a complete wbc count, differential and platelet counts, coagulation profile, and measurement of serum glucose, creatinine, BUN, phosphate, calcium, total protein, albumin, bilirubin, electrolytes, and lactate dehydrogenase.

Treatment was initiated in all patients between October 1, 1979, and March 1, 1981 (table 1). Four patients were females and 25 were males. A cystectomy was performed in only eight patients (27%) prior to pelvic recurrence and eventual referral for therapy. The mean age of the treated population was 60.4 years (median, 63; range, 39–74). Twenty-two patients had obstructive uropathy, while six had edema of the lower extremities in addition to the uropathy. Sixteen patients (55%) had received primary radiation therapy prior to referral. The clinical presentations of the treated patients are outlined in table 2. The patients were treated by the Departments of Medicine, Urology, and Diagnostic Radiology at The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute. Pathologic specimens were reviewed by the Department of Pathology and were classified by histologic subtype.

TABLE 1.—Pretreatment patient characteristics

Characteristics	No. of patients
Total in study	29
Sex	
Males	25
Females	4
Age (yrs)	
Median: 63	
Mean: 60.4	
Range: 39–74	
Prior radiation therapy	16 (55%)
Prior cystectomy	8 (27%)
Edema, lower extremities	6 (20%)
Obstructive uropathy	22 (76%)

The chemotherapy regimen consisted of three agents: 5-FU, doxorubicin, and mitomycin. Doxorubicin and mitomycin were each administered iv on 2 consecutive days. Doxorubicin (25 mg/m^2) was administered on Days 1 and 2 of each course, while mitomycin (5 mg/m^2) was administered iv on Days 3 and 4.

Bilateral hypogastric catheters were placed by the Department of Radiology on Day 1 of each regimen. The catheter placement was verified by radionuclide angiography, which enabled us to determine the proportion each catheter was responsible for infusing in the clinically determined tumor bed. This allowed us to divide the total 5-FU dose into each hypogastric catheter by the amount each was responsible for infusing in the tumor bed. Criteria for administration of each course were a granulocyte count $\geq 2000/\text{mm}^3$, a platelet count $\geq 100{,}000/\text{mm}^3$, and the absence of clinical, echocardiographic, radiographic, or electrographic changes suggesting doxorubicin-induced cardiomyopathy.

The patients initially received three courses of combined intra-arterial and systemic chemotherapy at a minimum interval of 4 weeks. Each patient then received the iv combination regimen at increasing time intervals, which were determined by marrow recovery.

Survival curves were prepared by the Kaplan-Meier method, and comparison between curves was

TABLE 2.—Clinical presentations of 29 patients

Presentation	No. of patients (%)
Pulmonary metastasis	2 (6.8)
Unilateral hydronephrosis	15 (52)
Bilateral hydronephrosis	7 (24)
Edema, lower extremities	6 (20)
Renal failure (creatinine > 2 mg/100 ml)	5 (17)
Liver metastasis	1 (3)
Rectal invasion	5 (17)
Bone metastasis	1 (3)

TABLE 3.—Responses by histologic subtype

Histology	No. of patients	
	Total	Responding (%)
TCC*	20	12 (60)
Adenocarcinomatous transformation (TCC)	1	1
Squamous transformation (TCC)	3	0 (0)
Adenocarcinoma†	4	4
Spindle variant	1	0
Total	29	17 (58)

*TCC = transitional cell carcinoma.
†Signet ring; mucin producing.

made with the Gehan modification of the Wilcoxon two-tailed test. Response criteria were simple. Patients who achieved an objective decrease in tumor size, as determined by radiographic criteria or by examination under anesthesia, were considered to have responded. Four of the 17 patients who responded had a decrease in tumor size, as shown by computed axial tomographic scan. One patient had relief of a small bowel obstruction, which was demonstrated by an upper gastrointestinal series; two had a decrease in urinary obstruction, as demonstrated by iv pyelogram; two had a marked improvement or disappearance of an infiltrating penile mass; and one had disappearance of neoplastic vaginal deposits. Each of the remaining seven responding patients had improvement or disappearance of disease, as shown by rectal or pelvic examination and cystoscopy. Repeat cystoscopy was performed in six patients, and four had disappearance of all measurable disease.

RESULTS

Seventeen patients (58%) achieved an objective response. The response rates by histologic subtype are shown in table 3. Twelve (60%) of 20 patients with transitional cell carcinoma responded. All four of the patients presenting with one of the adenocarcinoma subtypes responded, whereas the three patients with squamous transformation of transitional cell carcinoma failed to respond. The patient with a spindle variant failed to respond, while the patient with adenocarcinomatous transformation of a transitional cell carcinoma responded.

Four patients had distant visceral metastases in addition to local pelvic disease. One of the two patients with lung metastases responded with a > 50% reduction of a pleural-based nodule. A patient with liver metastases was treated with hepatic infusion of 5-FU after maintaining stable disease during the pelvic infusion of chemotherapy. The patient with bone metastases failed to respond.

Of the six patients who had repeat cystoscopic examinations, four had no evidence of residual local tumor, and two had persistent viable malignancy. Of the four with negative cystoscopic examinations, two were only visually inspected and two had negative biopsies in the previous tumor bed. Two of the patients with negative cystoscopic examinations eventually failed with hepatic metastases and upper para-aortic and supraclavicular nodal disease, without developing recurrent pelvic disease. Of the two patients who did have residual cancer on repeat cystoscopy, one had multiple superficial tumors, while the second had residual tumor in a persistent mass that was reduced > 50% in size at 1 year following initial treatment.

A third patient developed a second primary adenocarcinoma of the lung with no evidence of recurrent bladder cancer at 16 months following initial therapy.

Toxic effects are shown in table 4. One patient died as a result of chemotherapeutic toxicity. Death was due to respiratory failure, which at post-mortem examination was attributed to a previously unsuspected mitomycin lung toxicity. A second patient developed clinical mitomycin-induced interstitial pneumonia and responded to steroid therapy. One patient died in status asthmaticus following completion of two courses of infusional therapy. This

TABLE 4.—Toxic effects of intra-arterial chemotherapy for malignant urothelial tumors

Toxic effect	No. of patients (%)
Chemotherapy-related deaths	1 (3)
Motor neuropathy	4 (14)
Localized inflammatory skin changes*	29 (100)
Leukopenic fever	17 (59)
Mitomycin lung toxicity	2 (7)
Catheter placement complication†	3 (10)
Stomatitis	11 (38)

*Erythematous eruption in the distribution of the gluteal vessels. 9 patients (11%) had bullae skin changes.
†Arterial embolus, subintimal hemorrhage.

patient had a prior history of asthma, and his death was not attributed to the chemotherapy.

Inflammatory skin changes were encountered in the distribution of the gluteal vessels. The inflammatory skin changes, which were attributed to 5-FU, increased in severity with the number of courses infused. Most of the patients had painful erythematous reactions, while nine had localized bullous changes, which eventually resulted in superficial desquamation.

The sites of cutaneous toxicity could be predicted by radionuclide angiography. The drug concentration via the catheter that demonstrated the most cutaneous uptake was decreased where possible without compromising the drug delivered to the target site. Patients received meticulous care of the skin lesions, with frequent use of bactericidal cream (silver sulfadiazine, Silvadene) during the erythematous phase of cutaneous toxicity. If bullae developed, wet-to-dry soaks with 1:40 Burow's solution were used liberally. The use of air mattresses and frequent bedding changes avoided further deterioration in the drug-induced skin changes. No permanent skin ulceration was encountered, although frequently there were areas of residual hyperpigmentation at the site of prior cutaneous toxicity.

Three patients had complications of catheter placement. All patients received further intra-arterial chemotherapy, and each recovered with conservative medical management.

The survival time of the 17 responding patients was compared to that of the 12 nonresponding patients. The median survival of the responding patients was 52 weeks, while that of nonresponding patients was 28 weeks (fig 1). The median survival of patients with hydronephrosis was 32 weeks compared to 47 weeks for those without hydronephrosis ($P = 0.05$). The most profound negative effect on survival was the combined presence of hydronephrosis and edema of the lower extremities (fig 2).

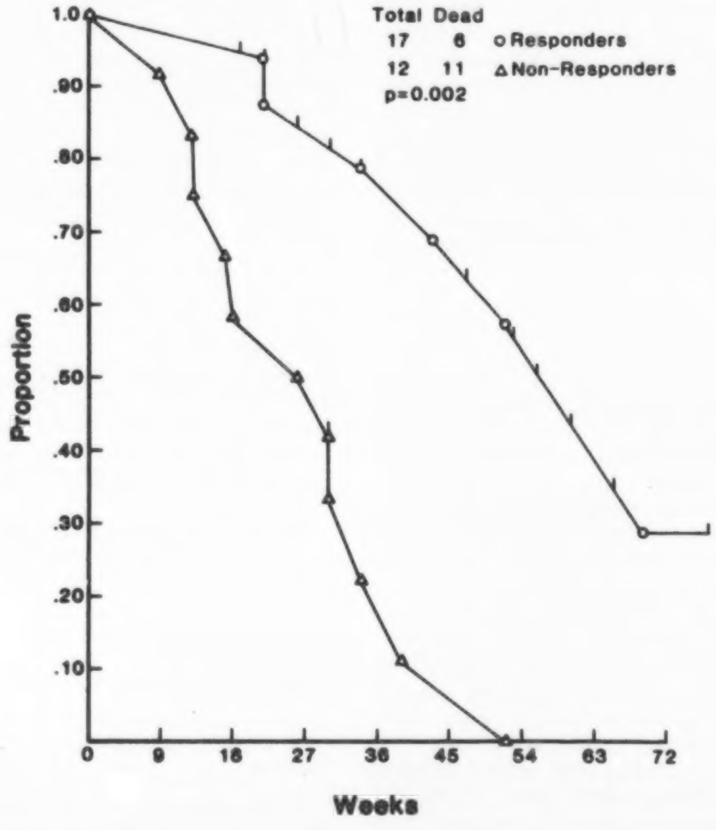

FIGURE 1.—Survival comparison of responding to nonresponding patients.

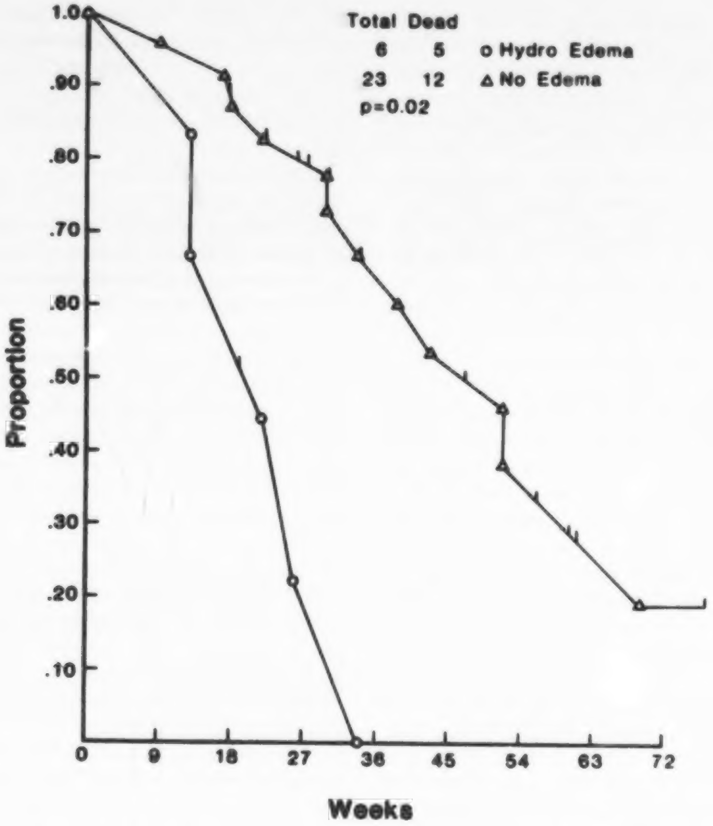

FIGURE 2.—Survival comparison of patients presenting with hydronephrosis and edema of the lower extremities (○) and the remaining population (△).

DISCUSSION

Pelvic recurrences of malignant urothelial tumors are among the most difficult and poorly tolerated clinical circumstances in genitourinary oncology. Symptoms usually result from malignant invasion of the sacral plexus, external genitalia, or urinary bladder.

Cisplatin is reported to be the single most active agent in the treatment of transitional cell carcinomas (10). Its use is often restricted because of renal insufficiency in this population with advanced age and the frequent complication of obstructive uropathy. Mitomycin, a modestly nephrotoxic agent, has previously been reported to be active in the treatment of bladder carcinoma. Early et al (11) reported a 21% response rate in 1973, and varying response rates ranging from 10% to 33% have been reported (12). 5-FU has been reported to be active as a single agent when administered iv. The most gratifying responses with 5-FU in the treatment of bladder carcinoma have been seen when it is used intra-arterially (13). Response rates with doxorubicin have varied greatly in different reports, with initial studies reporting a 38% objective response rate (14,15).

In the treatment of these select patients who required rapid local control of pelvic symptoms, we adopted a combined intra-arterial and systemic chemotherapy regimen. 5-FU was chosen as the single agent to be administered intra-arterially at a maximum tolerated dose.

The hypogastric arterial system, which is responsible for the arterial supply to the true pelvis, is readily approachable with the use of percutaneously placed arterial catheters. Nevin et al (13) have documented the effectiveness and toxicity of this approach using 5-FU in combination with irradiation. In our study, serious toxicity could be attributed to catheter placement, leukopenia, 5-FU infusion, or the idiosyncratic lung toxicity associated with mitomycin. Catheter placement in the hands of our Department of Diagnostic Radiology has been associated with a relatively small complication rate. In 78 catheter placements, three patients had complications directly related to the arterial catheters. Two of the patients had subintimal injections into their hypogastric arteries, with partial occlusion of the involved vessel, while one patient had an embolus to the left lower extremity. All patients were treated conservatively and received further infusion without significant complications. The high rate of leukopenic fevers (59%) can be attributed to the simultaneous presence of localized inflammatory skin changes and leukopenia. Four patients had 5-FU-induced transient motor neuropathy; all of these patients recovered completely. The one chemotherapy-related death was a result of respiratory failure; the patient died of mitomycin-induced interstitial pneumonia. The 29 patients received 1000 mg/m^2 of 5-FU intra-arterially for 5 consecutive days, which is the maximum tolerated dose. The dose-limiting toxic effect of this combined iv and intra-arterial regimen is the cutaneous inflammation attributed to local 5-FU toxicity.

The effectiveness of the combined intra-arterial and iv chemotherapy is evident when the survival durations of responding versus nonresponding patients are compared (fig 1). The median survival of the responding patients was 52 weeks, whereas the nonresponding patients had a median survival of 28 weeks, a significant difference ($P = 0.002$). The extent of disease greatly influenced the effectiveness of chemotherapy and the ultimate duration of survival. The patients with hydronephrosis had a significantly shorter survival than the remaining population ($P = 0.05$). We regard edema of the lower extremities and hydronephrosis as evidence of extensive local disease. The median survival in these patients was only 20 weeks and was not favorably influenced by the chemotherapy.

The patients who benefited most from the chemotherapy had initially presented without evidence of edema of the lower extremities. These patients had only local pelvic or small para-aortic nodal disease. Five of these patients had a repeat cystoscopy because they had no remaining measurable disease. One patient underwent cystoscopy for a radiographic abnormality thought to be a residual scar. Four of these six patients were found at cystoscopy to have no viable cancer, while two have active carcinoma, as shown by biopsy. Of the four with negative cystoscopic examinations, two developed distant metastatic disease while maintaining local control and two had no evidence of recurrent disease at 9 and 13 months. Of the two patients with residual carcinoma, one had multiple superficial tumors, while the second had a > 50% reduction in the size of his pelvic tumor and is asymptomatic on continued chemotherapy at 14 months following initial therapy.

The influence of histologic subtypes on eventual chemotherapeutic response is striking despite the small number of patients. Our data, as well as those of Nevin et al (13), strongly suggest that 5-FU is an active agent in adenocarcinoma of urothelial origin. The lack of any response among the three patients with squamous transformation of a transitional cell carcinoma suggests resistance of this histologic subtype to 5-FU-based chemotherapy.

Our experience with this select population demonstrated the effectiveness of intra-arterial 5-FU with simultaneous doxorubicin and mitomycin iv for the palliation of pelvic symptoms. Use of this 5-FU-based regimen is indicated in patients with adenocarcinomas of the urachus or urothelium and patients who either cannot receive cisplatin or have failed cisplatin-based regimens (16).

The ability of this combination to achieve a long-term response with durable control of local pelvic disease suggests a future role for pelvic infusional chemotherapy in the treatment of bladder cancer. Further studies are required to determine how best to integrate hypogastric infusional therapy, surgery, radiation therapy, and systemic chemotherapy in the treatment of bladder cancer.

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Phase I Study of Indicine *N*-Oxide in Patients With Advanced Cancer[1,2]

Takao Ohnuma,* Kasi S. Sridhar, Lynn H. Ratner, and James F. Holland[3]

SUMMARY

Indicine *N*-oxide is a pyrrolizidine alkaloid isolated from *Heliotropium indicum*, one of the widely used herbs in Ayurvedic medicine. Thirty-seven patients with solid tumors received the drug: 15 men and 22 women (mean age, 53 years). All had had prior chemotherapy, and 25 had had prior radiotherapy. Eighty-four percent had a performance status of 0–3 (Cancer and Leukemia Group B criteria). The drug was given as a short infusion over 15 minutes and repeated with a median interval of 4 weeks. Doses were escalated from 1 to 9 g/m². A total of 55 courses were evaluable. Dose-limiting toxic effects were leukopenia and thrombocytopenia, and the toxicity was cumulative with repeated doses. Other toxic effects included nausea and vomiting, anemia, and hepatic dysfunction. The hematologic toxicity tended to be more pronounced in patients with hepatic dysfunction, poor marrow reserve, and heavy prior chemotherapy and radiotherapy. There were no complete or partial responses. One patient with skin melanoma and another with ovarian carcinoma had improvement lasting 2 months. The maximally tolerated dose is 9 g/m² in our population. A recommended dose for therapeutic study is 7 g/m². High-risk patients should be started at a dose of 5 g/m². The treatment may be repeated at 4-week intervals with close monitoring of wbc and platelet counts. Dose reductions may be necessary for repeated courses.

[Cancer Treat Rep 66:1509–1515, 1982]

Indicine *N*-oxide is a water-soluble pyrrolizidine alkaloid isolated from *Heliotropium indicum* (1). This is one of the most widely used herbs in Ayurvedic medicine in India and in some other countries. Pyrrolizidine alkaloids were generally shown to be hepatotoxic; the digestion of such alkaloids by grazing animals resulted in chronic liver disease. This observation led to the study of their chemical and toxicologic properties. While the alkaloids produced antimitotic and mutagenic activity, as well as chromosomal breakage indicative of possible antitumor activity (2,3), the hepatotoxic effect of the compound dampened the enthusiasm for their clinical trials (3,4). Indicine *N*-oxide produced little hepatotoxicity in rodents and was active against a variety of experimental animal tumors including P388, L1210, and P1534 leukemias, B16 melanoma, and Walker 256 carcinosarcoma (1,5). It showed a route and schedule dependency. Thus, in the P388 system, the greatest degree of activity was provided by daily ip treatments for 9 consecutive days (q1d Days 1–9).[4] This schedule was ineffective by sc or oral administration of the drug. Given orally, the *N*-oxides of pyrrolizidine alkaloids produced toxic-

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[1] Received Oct 28, 1981; revised Feb 1, 1982; accepted Feb 11, 1982.

[2] Supported in part by contract N01-CM-97275 from the Division of Cancer Treatment and Public Health Service grant CA-15936 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Chemotherapy Foundation, Inc, New York, NY.

[3] Department of Neoplastic Diseases, Mount Sinai School of Medicine, New York, NY.

**Reprint requests to:* Dr. Takao Ohnuma, Department of Neoplastic Diseases, Mount Sinai School of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029.

[4] Clinical brochure: indicine *N*-oxide (NSC 132319). Prepared by the National Cancer Institute, Bethesda, MD, Sept 1977.

ity comparable to that of the free base, presumably because of reduction of the drug to the free base by the intestinal flora and subsequent metabolism to reactive pyrrolic compounds, the dehydropyrrolizidines, by the liver (6–8).

Toxicologic studies in large animals revealed the following dose-related and reversible organ system toxic effects: bone marrow hypoplasia with decreased wbc and rbc precursors; gastrointestinal toxic effects including anorexia, emesis, and hemorrhagic diarrhea; renal dysfunctions with elevated BUN and creatinine and nephrosis; and hepatic disorders manifested by elevated SGOT and SGPT and sulfobromophthalein (Bromsulphalein) retention (9,10).[4] Histopathologic evaluation of liver sections was, however, reported to be negative. Doses given within a short time interval were more toxic than equivalent doses given over a longer time. In dogs given a single dose, toxic dose low (TDL), toxic dose high (TDH), and lethal dose (LD) were 3, 12, and 24 g/m^2, respectively. On a daily × 5 schedule, lower total doses were tolerated.

Indicine *N*-oxide is the first pyrrolizidine alkaloid to be studied as an antitumor agent in humans. In concert with the effort by the National Cancer Institute (Bethesda, MD), three different schedules were explored by different institutions: single dose every 3–4 weeks (11,12), weekly dose (11), and daily dose for 5 consecutive days (13–15). Earlier clinical data have been reviewed (16). The present report describes our clinical experience (12) with the compound administered as an intermittent single iv bolus in patients with advanced cancer.

PATIENTS AND METHODS

Indicine *N*-oxide was supplied by the National Cancer Institute as a lyophilized powder, 1 g in a 20-ml vial. The drug was stored under refrigeration. The initial dose of 1 g/m^2 as a single iv injection was selected on the basis of large-animal toxicologic data (9,10) corresponding to ⅓ TDL in beagle dogs. Immediately prior to use, 1 g of the drug was dissolved in 20 ml of 5% dextrose in water or normal saline and administered iv as a rapid infusion over 15 minutes.

There was spaced entry of patients to minimize the risk of toxicity for each subsequent patient. After observing the course of three patients at the initial dose level, subsequent cohorts were entered at progressively higher dose levels, guided by a modified Fibonacci search scheme. At the dose level that produced moderate but non-dose-limiting toxicity, additional patients were entered. In the absence of dose-limiting toxicity, the dose was escalated in subsequent groups of patients as well as in subsequent courses for the same patients.

Criteria for entry in the clinical trials included the following: histologic diagnosis of metastatic cancer refractory to conventional therapy; adequate hematologic function with a peripheral wbc count $\geq 4000/\mu l$ and a platelet count $\geq 100,000/\mu l$; normal renal function (BUN $\leq$ 30 mg/dl and creatinine $\leq$ 1.5 mg/dl); adequate hepatic function (bilirubin < 1.2 mg/dl and hepatic transaminases < 50 milliunits/ml). In the latter phase of the study, patients with mild pre-existing hepatic and renal dysfunctions, as well as those with fluid accumulation causing a moderate third space, were included at lower doses to evaluate the influence of such dysfunctions on drug effects and vice versa. They included four patients with creatinine in the range of 1.7–2.1 mg/dl, three with mild liver dysfunction (SGOT $\leq$ 93 milliunits/ml, SGPT $\leq$ 74 milliunits/ml, and bilirubin < 1.2 mg/dl), and seven with ascites and/or $\geq$ 2+ leg edema constituting third-space fluid. Baseline studies included physical examination, body height and weight, cbc and complete automated chemical profile (SMA-6 and SMA-12), urinalysis, chest X-ray, and measurement of tumor lesions. Informed consent was obtained from all patients. Therapeutic effects were evaluated using our standard criteria (17).

RESULTS

Thirty-seven patients (15 men and 22 women) with advanced cancer were included in the study. Their ages ranged from 21 to 75 years (median, 55; mean, 53). All had received prior chemotherapy, and 25 had also received radiation therapy prior to entry in the study. The spectrum of diseases treated is listed in table 1. The patients were entered in the study after reversible toxic effects (except alopecia) from prior therapy had subsided. Their performance status (Cancer and Leukemia Group B criteria) was 0 (normal performance) in four patients, 1+ (ambulatory) in seven, 2+ (< 50% of waking time in bed) in ten, 3+ (> 50% of waking time in bed) in ten, and 4+ (100% bedridden) in six.

Three of the 37 patients entered were lost to followup for evaluation of toxic effects. Thirty-four patients received 55 courses, 14 of whom received subsequent courses. The median interval between courses was 26 days (range, 20–48). All of the 37 patients were analyzable for immediate side effects, two of whom died in < 2 weeks (on Days 10 and 12). Both patients had disease progression, and neither death was judged clinically to be drug-related. Delayed toxic effects were analyzed in 32 patients who were observed for 2 weeks to > 3 months.

The major toxic effect was myelosuppression. Dose

TABLE 1.—Spectrum of diseases treated with indicine *N*-oxide

Disease	No. of patients
Breast carcinoma	7
Colon carcinoma	4
Lung carcinoma*	4
Squamous cell carcinoma, head and neck	4
Sarcoma†	4
Renal cell carcinoma	2
Adenocarcinoma, salivary glands	2
Melanoma	2
Mesothelioma	2
Adenocarcinoma, ethmoid sinus	1
Gallbladder carcinoma	1
Ovarian carcinoma	1
Esthesioneuroblastoma	1
Transitional cell carcinoma, kidney	1
Adenocarcinoma, unknown primary	1
Total	37

*Squamous cell carcinoma, 2; adenocarcinoma, 1; and oat cell carcinoma, 1.

†Chondrosarcoma, 2; osteosarcoma, 1; and Ewing's sarcoma, 1.

escalation and occurrence of hematologic toxicity are summarized in table 2. While the degree of myelosuppression was progressively more pronounced at higher doses, severe leukopenia and thrombocytopenia were noted sporadically at intermediate doses. Thus, at a dose of 5 g/m^2, 13 courses of treatment were well-tolerated with no myelosuppression; however, one patient who had tolerated a dose of 3.3 g/m^2 without myelosuppression developed severe leukopenia and thrombocytopenia after a second course, given on Day 49 at a dose of 5 g/m^2. His wbc count nadir was 550/μl and his platelet count nadir was 14,000/μl. He remained leukopenic (< 4000 cells/μl) for 5 days and thrombocytopenic (< 100,000 cells/μl) for 21 days. He became febrile and received iv antibiotic therapy and a wbc transfusion. He also developed hepatic dysfunction with maximal bilirubin of 5.9 mg/dl, SGOT of 74 milliunits/ml, and SGPT of 75 milliunits/ml, which returned to normal by Day 34 (table 3). At a dose of 7.0 g/m^2, four of 16 initial courses and two of eight second courses were accompanied by severe myelosuppression (wbc count < 2000/μl and/or platelet count < 50,000/μl). Of these six courses, five were in patients with bone marrow or hepatic dysfunctions, the latter either pre-existing or drug-related. At a dose of 9 g/m^2, two of three patients had wbc count nadirs < 1000/μl and platelet count nadirs < 30,000/μl. They became febrile and were treated with iv antibiotics. One patient developed petechiae and required a platelet transfusion. With these observations, we concluded that we had reached the maximally tolerated dose in our patient population. The time to reach the nadirs of wbc and platelet counts did not differ between initial and subsequent courses. The duration of leukopenia and thrombocytopenia tended to be longer in subsequent courses. Anemia in the absence of bleeding or hemolysis was also observed more frequently at higher doses.

Evaluation of the influence of various risk factors on hematologic toxicity is summarized in table 4. Patients with heavy prior chemotherapy and radiotherapy, prior exposure to nitrosourea, decreased bone marrow reserve, or hepatic dysfunction appear to have developed severe hematologic toxicity.

Fourteen patients who received repeated courses of the drug were studied. Seven developed leukopenia and/or thrombocytopenia (table 5). In three patients, a repeated course at the same dose was accompanied by progressive lowering of the wbc count, and in two patients an escalation of the dose from 3.3 to 5 g/m^2 was accompanied by unexpectedly severe hematologic toxicity. These data indicate cumulative effects of the drug on the severity of myelosuppression.

Hepatic toxicity was noted in seven of 55 courses; there was mild pre-existing abnormal liver function in three patients (table 3). Hepatotoxicity is characterized by transient twofold (median) increases in basal SGOT, SGPT, and bilirubin, with the peak occurring on Day 19 (median), often accompanied by severe hematologic toxicity. Hepatic dysfunction reverted to normal in four courses within 10 days; two of the patients had pre-existing hepatic dysfunction. Of the patients who received the other three courses, one was lost to followup 21 days after treatment, one died on Day 14 of followup, and one died on Day 23 of followup.

Other toxic effects were of less clinical significance. Mild to moderate nausea and vomiting occurred in approximately one third of the courses given at a dose > 3.3 g/m^2. Transient diarrhea lasting 1 or 2 days occurred after two courses. Four episodes of low-grade fever were associated with the drug administration occurring on Days 2 and 4. Of the four patients with mild renal dysfunction (serum creatinine, 1.7–2.1 mg/dl) treated at doses of 2.0–7.0 g/m^2, neither deterioration of renal function nor any augmented hematologic toxicity was noted. Similarly, the presence of third space did not influence the degree of hematologic toxicity.

None of the patients had a complete or partial response. One patient with ovarian carcinoma had an improvement (less than a partial response) for approximately 2 months with the initial dose of 3.3 g/m^2 and the second dose of 5 g/m^2. Another patient with skin melanoma showed an improvement lasting

TABLE 2.—Hematologic toxicity of indicine *N*-oxide

Dose (g/m^2)	No. of evaluable courses	Course		Fall in Hgb > 1.5 (g/dl)*	Wbc count			Platelet count		
					Median nadir, × 10^3 cells/µl (range)	Median No. of days to nadir (range)	Median No. of days < 4.0 × 10^3 cells/µl (range)	Median nadir, × 10^3 cells/µl (range)	Median No. of days to nadir (range)	Median No. of days < 10^5 cells/µl (range)
1.0	3	First	3	0	4.3 (3.5–12.1)	3 (2–6)	2 (2)	121 (117–260)	5 (3–6)	
		Subsequent	0							
2.0	3	First	2	0	6.4 (5.3–7.4)	4 (3–5)		300 (102–498)	14 (3–25)	
		Subsequent	1	0	7.1	4		288	4	
3.3	8	First	5	0	4.8 (3.6–10.8)	11 (7–18)	11 (11)	256 (120–462)	14 (4–24)	
		Subsequent	3	0	8.5 (8.2–14.8)	11 (4–18)		440 (429–462)	13 (7–19)	
5.0	14	First	5	0	7.1 (5.3–10.3)	7 (1–24)		285 (85–384)	20 (1–24)	7
		Subsequent	9	1	5.0 (0.55–11.4)	11.5 (7–25)	4 (4–6)	214 (14–350)	14 (6–24)	19 (15–23)
7.0	24	First	16	6	4.2 (0.98–12.2)	13 (4–16)	8 (1–27)	136 (10.5–398)	14 (6–23)	7 (6–13)
		Subsequent	8	3	4.3 (0.98–7.6)	13.5 (6–17)	17 (14–32+)†	108.5 (3–475)	13.5 (10–14)	17 (14–32+)†
9.0	3	First	3	2	0.9 (0.8–4.0)	11 (8–11)	5 (5)	29 (6–226)	12 (8–12)	4 (4)
		Subsequent	0							

*Patients with documented bleeding not included. No episode of hemolysis was recognized.
†Wbc and platelet counts did not recover during observation period of 32 days.

TABLE 3.—Hepatic dysfunction in patients treated with indicine *N*-oxide

		SGOT (milliunits/ml)		SGPT (milliunits/ml)		Bilirubin (mg/dl)					
Dose (g/m²)	Course No.	Pre-treatment	Post-treatment (Day)	Pre-treatment	Post-treatment (Day)	Pre-treatment	Post-treatment (Day)	Hepatic function returned to normal on Day—	Wbc count nadir (× 10³ cells/μl)	Platelet count nadir (× 10³ cells/μl)	Comments
3.3*	1	93	140 (27)	43	185 (22)	0.5	0.7 (22)	32	5.8	250	
5.0	2	34	74 (29)	6	75 (29)	0.8	5.9 (29)	34	0.55	14	
5.0	2	11	115 (19)	31	143 (19)	0.6	1.2 (19)	†	4.9	49	Died on Day 23 of followup
7.0*	1	44	77 (24)	67	94 (26)	0.8	1.9 (17)	27	1.6	13	
7.0*	1	54	100 (14)	57	102 (14)	0.9	2.2 (10)	†	1.8	18	Lost to followup after Day 21
7.0	1	5	115 (21)	6	109 (19)	0.4	0.9 (21)	27	12.2	299	
7.0	1	45	78 (10)	23	16 (10)	0.1	0.3 (10)	†	1.6	54	Died on Day 14 of followup

*Pre-existing hepatic dysfunction.

†Hepatic function did not return to normal during observation period.

TABLE 4.—Relative risk factors for severe hematologic toxicity

	Toxicity	
	Severe*	Moderate or none
No. of courses evaluable	11	44
No. of courses with—		
Median performance status	2.0	2.5
Hepatic dysfunction†	5 (45%)	2 (4.5%)
Prior nitrosourea	3 (27%)	6 (14%)
> 5 prior chemotherapeutic agents	7 (64%)	13 (30%)
Prior radiotherapy	9 (82%)	16 (36%)
3rd space	2 (18%)	8 (19%)
Mild renal dysfunction	1 (9%)	3 (7%)
Bone involvement	1	3
Bone marrow dysfunction	2‡	0

*Wbc count $< 2000/\mu l$ and/or platelet count $< 50{,}000/\mu l$.

†3 patients had transient exacerbation of pre-existing mild hepatic dysfunction; 4 had hepatic toxic effects after treatment (*see* table 3).

‡Documented bone marrow dysfunction; 1 patient had hypocellularity and another had metastatic tumor; both had wbc count $> 4000/\mu l$ and platelet count $> 100{,}000/\mu l$ prior to treatment.

TABLE 5.—Comparison of hematologic toxicity after 1st and subsequent courses of indicine *N*-oxide*

Patient No.	Performance status	Dose (g/m^2)	Given on Day—	Wbc count nadir ($\times 10^3$ cells/µl)	Platelet count nadir ($\times 10^3$ cells/µl)
6	3	3.3		4.8	120
		5.0	32	3.9	41
7	3	3.3		10.8	262
		5.0	49	0.55	14
9	4	7.0		10.0	284
		7.0	21	0.975	3
22	2	7.0		10.7	398
		7.0	27	3.1	99
23	3	7.0		7.9	160
		7.0	27	4.4	97
		7.0	31	2.9	103
24	2	7.0		2.0	24
		5.0	38	4.9	49
29	1	7.0		3.1	222
		7.0	30	5.3	330

*No hematologic toxic effects were seen in 7 patients who had repeated courses of the drug at doses of 1–7 g/m^2; the maximum dose of 7 g/m^2 was given for 3 courses.

2 months and received four courses at a dose of 3.3 g/m^2.

DISCUSSION

The present study established that the dose-limiting toxic effect of indicine *N*-oxide in an intermittent single bolus schedule is myelosuppression and that the maximally tolerated dose is 9 g/m^2. A dose of 7 g/m^2 is tolerated in patients who have not been heavily treated with chemotherapy with or without radiotherapy. Patients with pre-existing hepatic dysfunction, heavy prior chemotherapy and radiotherapy, or borderline marrow reserve have less tolerance for the drug and should be given an initial dose of 5 g/m^2.

Hepatic dysfunction coincided with the drug administration in seven courses. This may be related to dose and number of courses (table 3), but the number is too small to suggest any definite conclusions. Patients with hepatic dysfunction, either preexisting or subsequent to treatment, were more likely to have augmented myelosuppression. These data support hepatic involvement in the metabolism and/or excretion of indicine *N*-oxide. It has been suggested that while approximately 60% of the compound, after iv administration, is excreted in the urine as an unmetabolized form, the rest of the parent drug diffuses passively into the gut where the flora reduces the drug to indicine, a hepatotoxic form, which reaches the liver (18).

It has been reported that prior exposure to a nitrosourea is a major risk factor (13). While this appears to be true in our analysis, it is thought that the prior exposure to a nitrosourea cannot be singled out as the sole risk factor. Heavy prior chemotherapy, large doses of radiotherapy, and borderline marrow reserve due to prior chemotherapy and/or tumor involvement are all associated with severe hematologic toxicity.

In the daily × 5 schedule, a daily dose of 3 g/m^2 or a total dose of 15 g/m^2/course has been recommended (13). Tolerance of a larger dose by the daily × 5 schedule as opposed to a single bolus is in accord with animal data. In a weekly × 4 schedule, a weekly dose of 6 g/m^2 or a total dose of 24 g/m^2/course has been suggested (11). While no assessment can be made of the therapeutic advantage of one schedule over another, it appears that more of the drug can be administered per course with the daily × 5 or weekly schedule than with the intermittent iv schedule. The compound has been reported to have encouraging activity in patients with refractory leukemia receiving a daily × 5 schedule (15).

Delayed recovery of marrow stem cells was evident by severe myelosuppression after a repeated dose given as late as Day 49. It was reported that a daily × 5 schedule resulted in little cumulative effect on the plasma drug concentration by Day 5 (13). The observed marrow toxicity does not necessarily represent drug retention in hematopoietic stem cells but may, rather, indicate a sublethal injury to those cells not killed outright, which delays their recovery and makes the marrow vulnerable to a second dose. Patients given repetitive prolonged treatment with the drug require close monitoring of the hematologic toxicity.

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Phase I Clinical Evaluation of 9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride (Bisantrene)[1,2]

Boh-Seng Yap,[3,*] Hwee-Yong Yap,[3] George R. Blumenschein,[3] Agop Y. Bedikian,[3] Robert Pocelinko,[4] and Gerald P. Bodey[3]

SUMMARY

9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)-hydrazone]dihydrochloride (bisantrene) is one of a series of anthracenedicarboxyaldehyde compounds that has significant antitumor activity similar to that of doxorubicin in experimental mouse tumor systems. A phase I clinical study was conducted using a weekly × 3 schedule. Twenty-six patients with a wide variety of advanced malignant solid tumors received a total of 39 courses of the drug at doses ranging from 40 to 180 mg/m^2/week × 3. Leukopenia was the dose-limiting toxic effect. Other toxic effects included malaise, which was observed in 49% of the courses, nausea and vomiting in 36%, fever in 21%, and phlebitis in 21%. No responses were observed in this study. For phase II studies, using this schedule, a starting dose of 150 mg/m^2/week × 3 repeated every 4–5 weeks is recommended. In patients who have received minimal or no therapy, the starting dose should be 180 mg/m^2.

[Cancer Treat Rep 66:1517–1520, 1982]

9,10-Anthracenedicarboxyaldehyde[bis(4,5-dihydro-1*H*-imidazol-2-yl)hydrazone]dihydrochloride (bisantrene) is one of several anthracenehydrazone analogs that was developed in an effort to identify a compound with a wide spectrum of antitumor activity resembling that of the anthracycline antibiotics but devoid of cardiotoxicity. The anthracene compounds do not possess the amino sugar portion of the anthracycline molecule, which was suggested by Adamson (1) to be responsible for the cardiotoxicity of the anthracyclines. Bisantrene has demonstrated significant wide-spectrum antitumor activity against experimental mouse leukemias and solid tumors, including L1210 leukemia, P388 leukemia, Lieberman plasma cell tumor, B16 melanoma, Colon tumor 26, and Ridgway osteogenic sarcoma (2). However, bisantrene did not show any activity against the P388/Adria leukemia or the Lewis lung carcinoma test systems. Its spectrum of antitumor activity is, therefore, similar to that of doxorubicin, and it is more active than dihydroxyanthracenedione against the Ridgway osteogenic sarcoma.

Although the precise mechanism of action of bisantrene has not been defined, preliminary evidence suggests that it is a DNA-reactive agent (2–4). In L5178Y mouse lymphoma tissue culture cells, bisantrene was a potent inhibitor of both RNA and DNA synthesis. When compared on an equimolar basis, bisantrene was approximately three times more potent than doxorubicin in inhibiting the incorporation of ^{3}H-uridine into RNA and four times more potent in inhibiting the incorporation of ^{3}H-thymidine into DNA.

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[1] Received Feb 1, 1982; accepted Feb 24, 1982.

[2] Supported in part by a grant-in-aid from the American Cyanamid Co, Medical Research Division, Pearl River, NY.

[3] Departments of Developmental Therapeutics (*B.-S. Yap*, *A. Y. Bedikian*, and *G. P. Bodey*) and Medicine (*H.-Y. Yap* and *G. R. Blumenschein*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4] American Cyanamid Co, Medical Research Division, Pearl River, NY.

[*] *Reprint requests to:* Dr. Boh-Seng Yap, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

The target organs for animal toxicology studies in rats, dogs, and monkeys were the bone marrow, the gastrointestinal tract, and the lymphatic systems. Toxic effects have included excessive salivation, emesis, epitaxis, lacrimation, decreased food consumption, weight loss, bloody diarrhea, rapid breathing, distended abdomen, hematuria, tremor, hind-limb weakness, ataxia, and increases in body temperatures. Myelosuppression was the major hematologic abnormality in dogs and monkeys and affected the wbc count more than the rbc count. Changes noted in serum chemistry determinations included increased alkaline phosphatase, creatinine phosphokinase, SGOT, SGPT, BUN, creatinine, total bilirubin, glucose, cholesterol, and triglycerides. There was no evidence of alopecia or cardiotoxicity in these reports (2).

Because of the potential usefulness of bisantrene, a phase I clinical study was initiated at our institution to determine the maximum tolerated dose and toxicity associated with the administration of this compound on a weekly × 3 schedule.

MATERIALS AND METHODS

All adult patients who were entered in this study had histologic proof of advanced malignancy and were not candidates for known regimens or protocol treatments of higher efficacy or priority. An estimated life expectancy of at least 12 weeks was required, and all patients had measurable disease, although this was not a prerequisite for entry in the study. All patients were required to have adequate bone marrow function (defined as a peripheral absolute granulocyte count $> 2000/mm^3$ and a platelet count $> 100,000/mm^3$, with normal bone marrow cellularity), adequate renal function (creatinine < 2.5 mg/100 dl), and adequate liver function (bilirubin < 2 mg/100 dl). In addition, patients were required to have recovered completely from toxic effects of all previous chemotherapy or radiotherapy. Informed consent, indicating the investigational nature of this study, was obtained from all patients in accordance with federal and institutional policies.

Bisantrene was supplied by the American Cyanamid Company as a sterile lyophilized powder in vials containing 10, 80, or 200 mg of the free base of the compound with no preservatives. Bisantrene is a hygroscopic orange solid that is stable at room temperature. For clinical use, it was reconstituted with sterile water for injection (USP) and was further diluted in 250 ml of 5% dextrose in water or normal saline to be administered iv over 1 hour. The diluted orange-yellow solution was given within 2 hours of preparation.

A single dose was given weekly for 3 weeks followed by a 2-week observation period. Each course was repeated every 5 weeks. The toxic dose low in the single-dose studies in dogs was 64 mg/m^2 and one third of this dose is approximately 22 mg/m^2. However, based on phase I data available from Myers et al (5) when we began the study, we chose an initial starting dose of 40 mg/m^2. During the initial phase of the study, the drug dose was escalated by 100% until any signs of biologic activity were observed. Subsequent dose escalations were then made at 25%–50% increments until dose-limiting toxic effects were observed. At least three patients were studied at each dose level and were evaluated for at least 5 weeks before additional patients were given the next dose level. Dose escalation was allowed within patients.

During the study, each patient was closely observed and all toxic effects during courses of therapy were recorded. The patient's blood pressure, pulse, respiration, and temperature were monitored before and at the end of drug administration. Cbc with differential and platelet count were performed twice weekly and SMA-12 chemistry pattern screen, serum electrolytes, prothrombin time, partial thromboplastin time, and urinalysis were repeated at least once each week and prior to each course of therapy. Appropriate radiologic examinations and electrocardiograms were performed before treatment and repeated at least every 10 weeks. Treatment was discontinued when there was evidence of tumor progression following one or more courses of treatment. Patients were considered evaluable for toxicity if they received at least one course of therapy with 2 weeks of subsequent observation. Standard criteria of response were used to evaluate the antitumor effect of the drug.

RESULTS

Twenty-six patients (21 women and five men) were entered in this phase I study; the median age was 53 years (range, 32–75). Most of the patients had breast cancer (11 patients), but other tumor types included colon cancer (four); lung cancer (three); melanoma (three); sarcoma (two); and hypernephroma, pancreatic carcinoma, and carcinoma of unknown primary (one each). All patients had received prior chemotherapy and 50% had received three or more treatment regimens. Fourteen (54%) patients had been given radiation therapy. Approximately 85% of the patients had a Zubrod performance status of 1 or 2 and 15% had a score of 3 or 4. A total of 39 treatment courses of bisantrene were administered at escalating doses ranging from 40 to 180 mg/m^2/week × 3. The number of patients treated

Table 1.—Dose escalation scheme of bisantrene

Dose level (mg/m^2/wk × 3)	No. of patients	No. of courses		
		Initial	Subsequent	Total
40	4	4	1	5
80	6	3	4	7
120	9	5	6	11
150	9	7	3	10
180	6	6	0	6

Table 2.—Nonhematologic toxicity by dose of bisantrene

	Dose (mg/m^2)			
	80	120	150	180
No. of evaluable courses	7	11	10	6
No. of courses with—				
Malaise	1	5	7	6
Nausea and vomiting	2	3	6	3
Fever	0	0	4	4
Phlebitis	1	2	3	2
Anorexia	2	0	3	1
Hair loss	0	2	1	0
Diarrhea	0	1	1	0

and the number of treatment courses given at each dose level of bisantrene are shown in table 1.

Although all 26 patients were evaluable for acute toxic reactions, one patient died from progressive disease on Day 9 after having received two doses of Course 1 therapy. No drug-related toxic reaction was noted in any patient receiving < 80 mg/m^2/week × 3 (table 2). The most common side effect reported by the patients was general malaise accompanied sometimes by a flu-like illness. This usually occurred after the second dose of each course of therapy and became more pronounced after the third dose. Each episode lasted about 2–3 days. Nausea and vomiting, seen in approximately one third of the patients, were generally mild. Severe phlebitis at the site of infusion occurred in eight of 39 courses administered. However, 16 of the 39 courses were administered via the subclavian vein using a long-line catheter. Therefore, the true incidence of phlebitis was 35% in the courses administered via the peripheral veins. Hypotension was not observed, although it was reported by Myers et al (5). No evidence of hepatic, renal, or clinical congestive heart failure was observed in this study.

The myelosuppressive toxicity of bisantrene is shown in table 3. Leukopenia was the major dose-limiting toxic effect. At 150 mg/m^2, the median lowest wbc count was 1800/mm^3 and the median lowest absolute granulocyte count (AGC) was 900/mm^3. The median time to occurrence of the lowest recorded wbc count was 16 days, with full recovery by Day 28. Thrombocytopenia (platelet count $< 100{,}000$/mm^3) was not seen. At 180 mg/m^2, the median wbc count was 1400/mm^3 and the lowest median AGC was 400/mm^3. The median time to occurrence of the lowest recorded wbc count was 18 days, with full recovery by Day 29. A platelet count of 77,000/mm^3 occurred in one patient at this dose level. This patient had

Table 3.—Hematologic toxicity of bisantrene

Dose level (mg/m^2 wk × 3)	No. of evaluable courses	Lowest counts, cells × 10^3/mm^3								
		Wbc count			AGC			Platelet count		
		Day	Median	Range	Day	Median	Range	Day	Median	Range
40	5	18	3.5	2.0–5.1	15	2.1	0.9–4.0	8	244	88–288
80	7	21	4.2	2.3–5.6	21	1.9	1.1–3.8	18	268	119–325
120	11	18	2.3	1.3–4.1	22	1.3	0.6–2.4	8	262	180–533
150	10	16	1.8	0.8–3.1	17	0.9	0.5–0.2	14	203	125–365
180	5	18	1.4	0.5–2.9	18	0.4	0.2–1.2	20	189	77–410

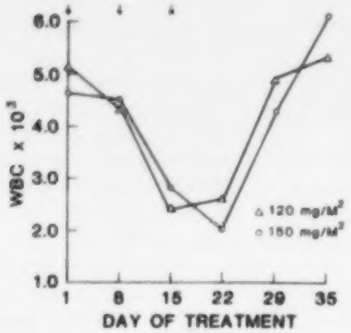

FIGURE 1.—Weekly median lowest wbc counts at 120 and 150 mg/m^2.

received extensive prior chemotherapy and radiotherapy. It was not possible to assess cumulative myelosuppression since only one patient had received more than three courses of therapy. Figure 1 shows the median lowest wbc count obtained weekly for courses at 120 and 150 mg/m^2, respectively. Maximal leukopenia occurred between Days 15 and 22 at both dose levels, with full recovery by Day 29, even though the third dose of the drug was given on Day 15.

While the major reasons for this study were to evaluate the dose and toxicity of bisantrene, observations were also made regarding antitumor activity. No objective tumor regression was observed in any of the 25 patients evaluable for response.

DISCUSSION

In this phase I clinical study, we have shown that bisantrene can be given safely to patients on a weekly × 3 schedule. The toxic effects of the drug are tolerable, predictable, transient, and reversible. The dose-limiting toxic effect was leukopenia, with very minimal effect on the platelet count. Since leukopenia is rapidly reversible (fig 1), it may be possible to administer bisantrene every 10–14 days without a rest period, as with the vinca alkaloids. The major nonmyelosuppressive toxic effect was phlebitis, occurring in 35% of the courses given via the peripheral veins. To circumvent the problem of phlebitis, we recommend the routine use of an indwelling central venous catheter, diluting the drug in 500 ml of dextrose in water or normal saline, and administering over 1 hour.

Other investigators have conducted phase I trials of bisantrene with single doses given every 3–4 weeks or daily for 5 days (5,6). The recommended dose for the single-day schedule is 260 mg/m^2 and the 5-day schedule is 80 mg/m^2/day. Although no responses were observed in our heavily pretreated patient population, it is encouraging to note that other centers have reported responses in hypernephroma, hepatoma, melanoma, adenocarcinoma of the lungs, and lymphoma. Bisantrene is, therefore, an attractive compound for further clinical studies. It has significant wide-spectrum antitumor activity in experimental animals, no cardiotoxicity in animal model systems or in this study, tolerable and reversible toxic effects, and antitumor activity in man. The results of this study suggest that for phase II studies of bisantrene, the starting dose should be 150 mg/m^2/week × 3 repeated every 4–5 weeks. In patients who have received minimal or no prior therapy, the starting dose should be 180 mg/m^2.

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Cyclophosphamide-Induced Cardiomyopathy in the Rat[1,2]

Harold A. Hopkins,[3,*] William L. Betsill, Jr,[4] Ann S. Hobson,[3] and William B. Looney[3,5]

SUMMARY

Cyclophosphamide given to ACI inbred rats produced histologic evidence of cardiomyopathy and degenerative vascular changes beginning as early as 4 days after the first of three 150-mg/kg doses (0.9 g/m^2). The interval between doses was 14 days to allow bone marrow to recover after each treatment. Twofold increases in heart weight and DNA content were observed 56 days after initial treatment; compensatory hypertrophy and a marked infiltration of the myocardium by lymphocytes were observed histologically at that time. Heart weight and DNA content were again at control levels on Day 126. Hydroxyproline content, which increased along with DNA and weight, did not decrease until after Day 196, confirming a morphologic finding of increased fibrosis after cyclophosphamide which eventually decreased in severity. These data re-emphasize the necessity for considering both the acute and chronic toxic effects, as well as recovery, when developing treatment schedules employing therapeutically effective chemotherapeutic agents.

[Cancer Treat Rep 66:1521–1527, 1982]

For the solid tumor 3924A in the ACI rat, combined therapy with cyclophosphamide and radiation is effective in producing up to 100% complete response, with ≥ 50% of these rats eventually being cured in some treatment groups (1). These schedules avoid death from acute toxicity to gut or marrow by allowing at least 11 days between drug treatments, this being the period of time necessary for recovery of bone marrow following an LD10 dose of cytotoxic drug [cyclophosphamide, 5-FU, or doxorubicin (2–4)]. To prevent recurrence of the tumor at the original site and to cure or arrest growth of metastases that were disseminated prior to local cure, a prolonged course of chemotherapy may be necessary. This may require scheduling around long-term changes in critical organs of the body, which may be different from those limiting therapy at the start of treatment.

We have initiated long-term studies (to about 1 year from treatment) to survey a number of organs (liver, gut, kidney, bone marrow, peripheral blood, spleen, heart, lung, thymus, and bladder) in the rat for damage which might limit the long-term use of cyclophosphamide in controlling metastatic growth or preventing recurrence of the primary tumor. This report summarizes our observations for the heart when ACI rats were given three doses of cyclophosphamide at 14-day intervals, each dose being the LD10 for a single dose. Parallel studies of bone marrow and peripheral blood were made to compare the time sequence of the acute effects and recovery of the hematopoietic system to the time sequence of the acute effects and recovery in the heart.

METHODS

Inbred female ACI rats (Laboratory Supply Co, Indianapolis, IN) weighing 120–140 g were given

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[1] Received July 21, 1981; revised Feb 16, 1982; accepted Mar 17, 1982.

[2] Supported in part by Public Health Service grant CA-20516 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Division of Radiobiology and Biophysics (*H. A. Hopkins* and *W. B. Looney*) and Department of Pathology (*A. S. Hobson*), University of Virginia School of Medicine, Charlottesville.

[4] Department of Pathology, Medical University of South Carolina, Charleston.

[5] We thank M. MacLeod, K. Lotts, J. Key, and S. Mays for their technical assistance.

**Reprint requests to:* Dr. H. A. Hopkins, Box 392, University of Virginia Hospital, Charlottesville, VA 22908.

three ip doses of cyclophosphamide (each 150 mg/kg) at 14-day intervals. Cyclophosphamide was supplied by Mead Johnson Research Center, Evansville, IN. Groups of three rats were killed 4 and 14 days after each individual treatment and at 5-week intervals thereafter for 1 year. One hour prior to killing, each rat was given 50 microcuries of [methyl-^{3}H]thymidine (^{3}H-TdR) ip (specific activity, 3 curies/mmol). At killing, blood for wbc count, hematocrit, and hemoglobin was taken by cardiac puncture under ether anesthesia. Hearts were removed, cut transversely through the ventricles, cleared of blood, and weighed. The interventricular (septal) aspects of the ventricles were used for DNA and hydroxyproline measurements. The lateral aspect of the left ventricle was reserved for histopathologic examination.

Specimens were fixed for histology in neutral, buffered, 10% Formalin; embedded in paraffin; sectioned at 6 μ; and stained with hematoxylin and eosin. Masson's trichrome stain was used to evaluate heart muscle for fibrosis. For autoradiographs, specimens were sectioned at 4 μ, prepared with Kodak NTB-2, exposed for 2 weeks, developed with Kodak D-19, and stained through the emulsion with hematoxylin.

DNA was extracted from tissue homogenates by heating at 90°C for 20 minutes with 5% trichloroacetic acid after first eliminating RNA and small molecules by alkaline hydrolysis, precipitating with cold acid, and washing the residue twice with cold 10% trichloroacetic acid. DNA was measured by the method of Burton (5), with calf thymus DNA (Sigma Chemical Co, St. Louis, MO) as the standard.

For hydroxyproline measurements, tissue was hydrolyzed by heating at 100°C overnight in 6 N HCl. Analysis was by the procedure of Switzer and Summer (6).

RESULTS

Deaths among ACI rats given 150-mg/kg doses of cyclophosphamide on Days 0, 14, and 28 were largely confined to the 70-day period following the start of treatment (fig 1). The survival rate in figure 1 is based on the original number of rats treated, without adjustment for animals killed during the experiment, and was approximately 70% at 70 days. The 70% survival rate at 70 days is consistent with each single 150-mg/kg dose being an LD10 for the ACI rat, without cumulative toxicity, for a total dose of 450 mg/kg in 28 days. Each dose of cyclophosphamide resulted in a loss of 85%–90% of the bone marrow cells, measured as milligrams of DNA per tibia, and even greater decreases in wbc counts, particularly after repeated treatment (table 1). However, both bone marrow and wbc counts were fully restored 14 days after each treatment.

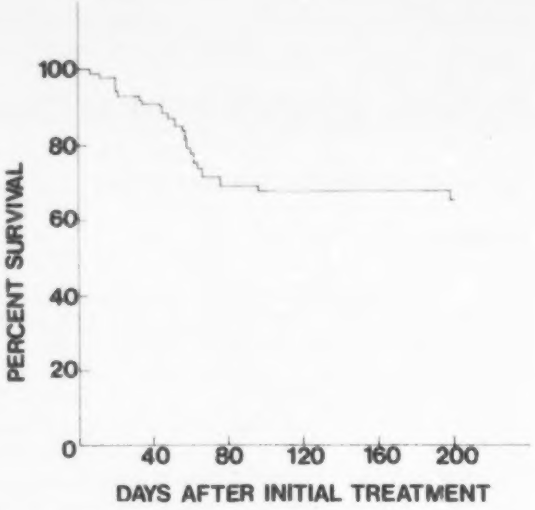

FIGURE 1.—% survival of ACI rats given 3 doses (150 mg/kg each) of cyclophosphamide on Days 0, 14, and 28.

Heart weight and DNA data for these ACI rats given three 150-mg/kg doses of cyclophosphamide are presented in figure 2. Heart weight did not change during the period of treatment but began to increase on completion of three courses of treatment; it was almost double that of untreated controls on Day 56. This increase in weight was paralleled by an increase in total heart DNA, signifying an increase in the number of nucleated cells. Histologic evaluation of these hearts revealed an influx of lymphocytes, which is the likely explanation for the DNA changes; both the lymphocytic infiltrate and the hypertrophic changes described below would contribute to the observed weight increases. Weight and DNA content of the heart returned to control levels on Day 127, and although not shown in figure 2, these were still at control values on Day 307. ^{3}H-TdR incorporation into total DNA increased shortly after the start of cyclophosphamide treatment and continued at approximately the same rate during the period of DNA accumulation, but returned to control values 91 days after treatment (table 2). Autoradiographs of hearts taken 56 days after the start of cyclophosphamide treatment revealed that nuclei of both muscle and nonmuscle cells were labeled. Labeled nuclei were rare in untreated hearts. Additional work will be needed to establish the importance of the ^{3}H-TdR labeling in cardiac muscle

Table 1.—Bone marrow and peripheral blood parameters after cyclophosphamide treatment of ACI rats

Days after initial dose	Total dose delivered (mg/kg)	Bone marrow DNA (mg/tibia)	Hemoglobin	Hematocrit	Wbcs	Lymphocytes	Polymorphonuclear leukocytes
0	0	0.45 ± 0.10*	13.1 ± 0.2	38.8 ± 0.2	3970 ± 750		
4	150	0.06 ± 0.002	11.6 ± 0.3	33.2 ± 0.4	530 ± 230		
14	150	0.68 ± 0.02	12.8 ± 0.4	38.3 ± 1.5	3600 ± 100		
18	300	0.06 ± 0.008	11.6 ± 0.4	35.7 ± 0.7	63 ± 13		
28	300	0.53 ± 0.04	12.1 ± 0.1	36.5 ± 0.5	4200 ± 320	1590 ± 350	2300 ± 290
32	450	0.05 ± 0.005	12.3 ± 0.4	36.0 ± 1.3	27 ± 6		
42	450	0.56 ± 0.01	12.5 ± 0.6	38.7 ± 2.2	8300 ± 2250	1810 ± 230	6160 ± 2450
56	450	0.49 ± 0.08	13.5 ± 0.9	43.0 ± 3.5	3130 ± 820	1820 ± 490	710 ± 135
91	450	0.42 ± 0.09	14.3 ± 0.2	44.7 ± 0.9	4670 ± 550		
126	450	0.20 ± 0.05	13.7 ± 0.6	42.0 ± 1.5	2770 ± 270	1400 ± 140	1130 ± 310
161	450	0.35 ± 0.07	12.6 ± 0.5	39.2 ± 1.6	2970 ± 1040		
196	450	0.14 ± 0.02	13.4 ± 0.3	39.2 ± 0.4	2530 ± 640		
231	450	0.29 ± 0.03	12.8 ± 0.4	40.0 ± 0.6	4230 ± 840		
266	450	0.28 ± 0.04	13.5 ± 0.1	41.7 ± 0.3	2100 ± 360		
307	450	0.28 ± 0.06	13.2 ± 0.2	42.0 ± 1.2	1830 ± 340		
Controls							
32	0	0.42 ± 0.04	13.3 ± 0.1	41.0 ± 0	3770 ± 200		
56	0	0.27 ± 0.08	13.6 ± 0.4	41.3 ± 1.4	3570 ± 380	2410 ± 470	780 ± 190
126	0	0.31 ± 0.03	13.7 ± 0.3	42.2 ± 0.6	3400 ± 100	1970 ± 53	1070 ± 86
196	0	0.33 ± 0.02	13.9 ± 0.0	42.0 ± 0.0	2920 ± 490		
307	0	0.50 ± 0.05	13.1 ± 0.6	42.0 ± 1.9	2300 ± 310		

*Mean ± SE for 3 animals.

cells subjected to cyclophosphamide treatment. As yet, no stimulus has been identified that will produce cardiac muscle cell proliferation in the adult heart, although interstitial cell proliferation does occur (7).

Only left ventricular tissue was available for light microscopic evaluation. Abnormal changes were noted at 4 days, the earliest time evaluated after therapy. These changes consisted of early degenerative vascular features including exudation of

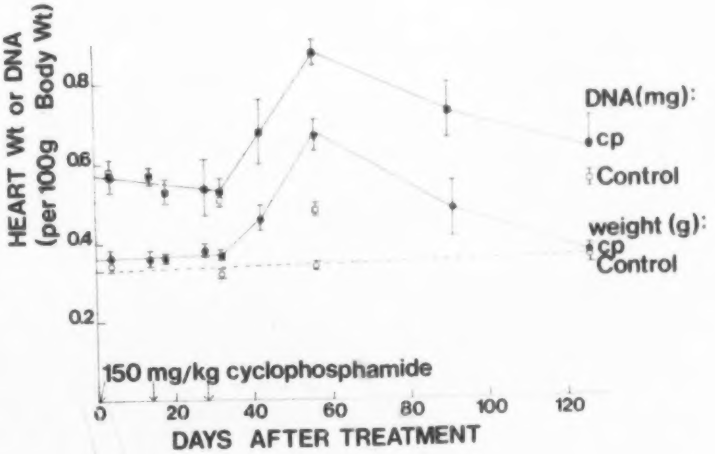

Figure 2.—Heart weight and total heart DNA content of ACI rats given 3 doses (150 mg/kg each) of cyclophosphamide. ■ = mg of DNA for rats given cyclophosphamide; □ = mg of DNA for control rats; ● = heart weight for rats given cyclophosphamide; ○ = heart weight for control rats. Maximum values after treatment for both weight and DNA were significantly different from control values ($P < 0.01$).

TABLE 2.—Incorporation of ^{3}H-TdR into heart DNA and hydroxyproline content of heart after cyclophosphamide treatment of ACI rats

Days after initial dose	Total dose delivered (mg/kg)	10^{-4} × dpm/mg DNA		µg of hydroxyproline/heart/100 g of body weight	
		Treated	Control	Treated	Control
0	0	—	1.85 ± 0.20*	—	186 ± 12.0
4	150	2.13 ± 0.38		180 ± 10.0	
14	150	4.27 ± 0.02		130 ± 6.7	
18	300	2.52 ± 0.22		183 ± 7.6	
28	300	8.59 ± 2.43		198 ± 14.0	
32	450	3.08 ± 0.51	1.37 ± 0.18	201 ± 4.6	171 ± 9.0
42	450	6.89 ± 1.80		307 ± 23.0	
56	450	4.82 ± 0.36	1.44 ± 0.05	410 ± 30.0†	180 ± 13.0
91	450	1.20 ± 0.29		318 ± 85.0	
126	450	1.54 ± 0.56	0.99 ± 0.11	320 ± 28.0†	227 ± 10.0
161	450	0.90 ± 0.10		405 ± 88.0	
196	450	1.26 ± 0.06	0.90 ± 0.18	414 ± 44.0†	224 ± 27.0
231	450	1.15 ± 0.07		—	
266	450	1.51 ± 0.22		321 ± 41.0	
307	450	0.88 ± 0.12	0.61 ± 0.06	241 ± 12.0	155 ± 21.0

*Mean ± SE for 3 animals.

†Different from control ($P < 0.05$).

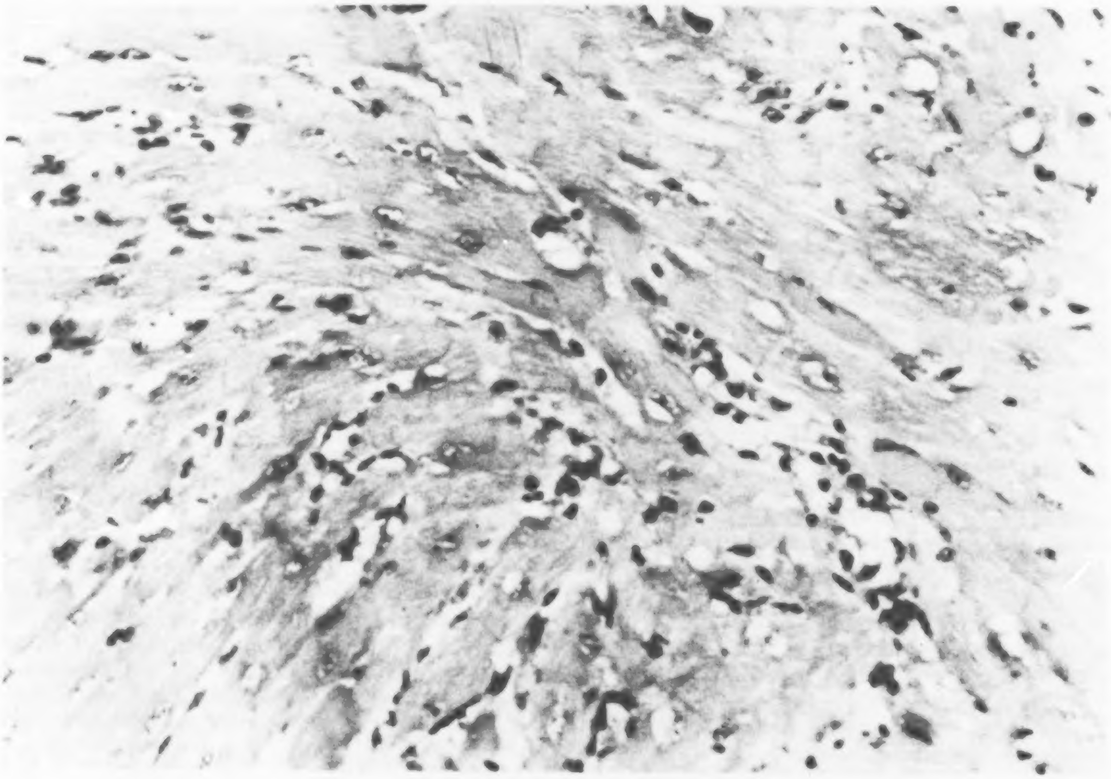

FIGURE 3.—Vacuolar degeneration of myocyte cytoplasm 56 days after start of cyclophosphamide treatment (hematoxylin and eosin stain, × 200).

lymphocytes into the perivascular wall and minimal tissue degeneration (vasculitis). Endothelial cells appeared hypertrophic, "picket-fencing" the luminal aspect of the vessel. This change was noted only focally in the left ventricular tissue and involved small arteries and arterioles. The epicardial portions of the coronary arteries were not available for evaluation. By Day 14, the endocardial surface and subendocardial connective tissues manifested vacuolar degeneration. Myocytic cytoplasmic vacuolization became noticeable at approximately Day 18. These changes increased in intensity and became more confluent. The degenerative features and a lymphocytic infiltrate increased in severity until Day 56 (fig 3). After this time the vacuolar myocytic changes appeared to lessen in severity. In actuality, markedly degenerated myocytes were replaced by early fibrosis with compensatory hypertrophy of adjacent, less-damaged myocardium. Hypertrophic changes in the adjacent myocardium were noted to a lesser degree in animals killed earlier in the experiment. Vascular changes (vascular sclerosis) noted at Day 4 progressed in severity (fig 4) and persisted throughout the extent of the experiment. Chronic tissue changes (eg, focal calcification and cartilaginous metaplasia) also increased in intensity with time for the earlier time points. However, there was less identifiable fibrotic tissue at 307 days than at either 56 or 91 days.

Hydroxyproline content of heart tissue exceeded control values on Day 42 after initiation of treatment (table 2). Twofold increases over control values were observed on Day 56 and generally persisted to Day 196, but diminished thereafter to approximately control values on Day 307. Essentially all of the hydroxyproline in animal tissues is found in collagen, the matrix substance of fibrosis in tissue (8). Thus, chemical analysis confirmed the morphologic finding of increased fibrosis after cyclophosphamide, followed by a recovery phase.

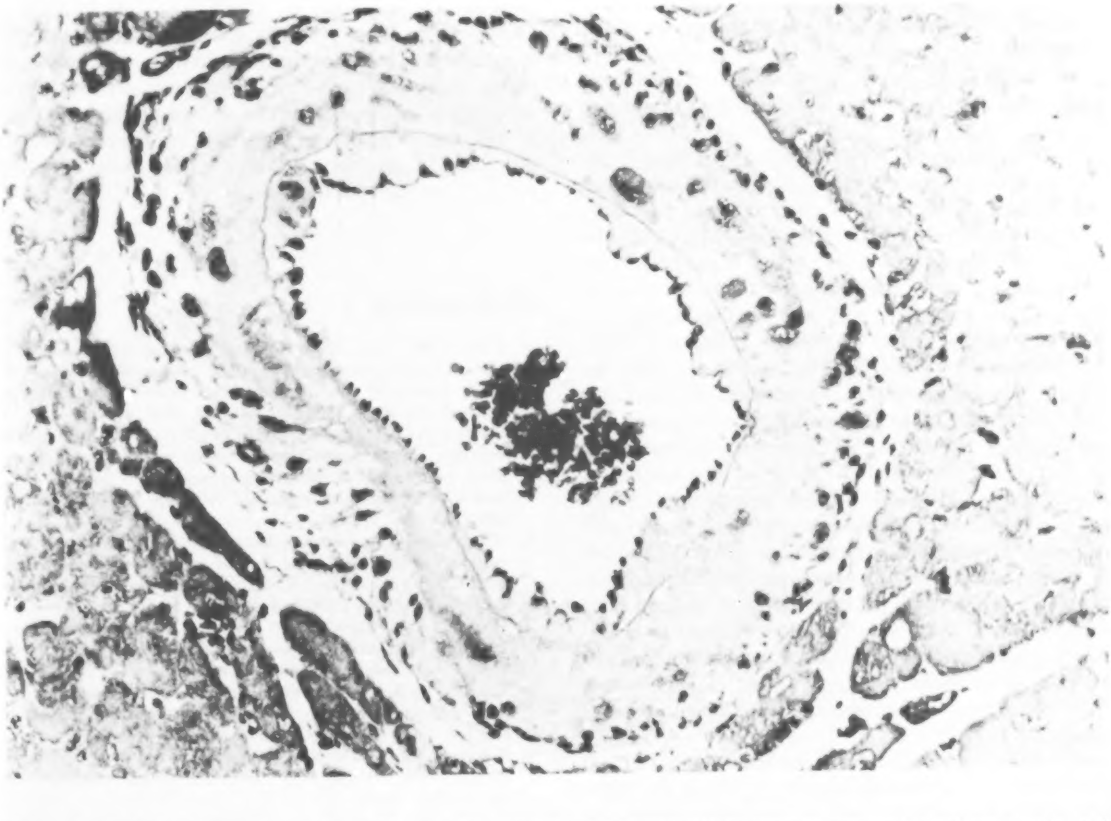

FIGURE 4.—Arterial degenerative change 126 days after start of cyclophosphamide treatment (hematoxylin and eosin stain, × 40).

DISCUSSION

The effectiveness of cyclophosphamide for therapy in man and experimental animals having a variety of neoplasms is well-established (9). The maximum dose tolerated by man is about 27–41 mg/kg (or 1.0–1.5 g/m^2), which can be repeated at 17–24-day intervals without producing cumulative hematologic toxic effects (10). With very high-dose cyclophosphamide of 40–50 mg/kg/day or more for up to 4 days, as used for intensive remission induction and bone marrow ablation prior to marrow transplantation, fatal cardiotoxic reactions have been observed (11–14). These high doses in man correspond on a surface area basis (15) to 6.7 g/m^2, for example, for the dose of 180 mg/kg in 4 days as used by Gottdiener et al (13). In contrast, the morphologic and compositional changes reported here for the ACI rat are occurring at a total dose of 2.7 g/m^2 delivered in three equal doses at 14-day intervals. This difference in total dose suggests either that the rat is more sensitive to cyclophosphamide than is man with respect to cardiac damage or that in man damage may be occurring that has so far escaped detection except in those cases where death has ensued. Some cardiac damage was observed in the rat after only one dose; however, none of the deaths that occurred could be attributed to cardiac failure.

The pathogenesis of cardiotoxicity from very high-dose cyclophosphamide therapy probably results from endothelial toxicity with transudation of the drug or its products into surrounding tissue (13,16). The morphologic changes noted here for the rat at doses corresponding on a surface area basis to conventionally used clinical doses are similar in several respects to those noted by others in human hearts after very high doses (12). The prominent vascular damage, the interstitial inflammation, and the myocytic degeneration in these animals all support the hypothesis of Buja et al (16) and of Gottdiener et al (13) regarding endothelial injury as the source of tissue damage. However, in our experiment, there was no evidence of focal hemorrhage, neutrophil infiltrate, and actual myofibrillar necrosis, which are present in acute human cyclophosphamide cardiotoxicity (17). The pathogenesis of the marked lymphocytic infiltration observed in the rat is unknown. Other drugs, especially catecholamines, are reported to produce changes which closely mimic those for cyclophosphamide cardiotoxicity in the human heart (17).

Mills and Roberts (14) have contrasted the clinical manifestations of anthracycline and cyclophosphamide cardiotoxic effects, both being dose-related but differing in that doxorubicin toxicity appears to be the cumulative result of myocardial injury, whereas the onset of cyclophosphamide cardiomyopathy is acute. Our data with the rat after relatively low total doses suggest that cumulative damage may occur with cyclophosphamide as well. A recent report (18) suggests that decreasing peak plasma levels of doxorubicin by continuous infusion over 1, 2, or 4 days reduces cardiac toxic effects without compromising antitumor activity. A similar modification in drug administration should be evaluated experimentally with cyclophosphamide, but a fatality has been reported in the clinic when a total dose of 120 mg/kg, the lowest cardiotoxic dose so far reported in man, was infused over a 24-hour period (12). Recent reports (19,20) demonstrate that cyclophosphamide toxicity that does not cause immediate death may be compensated, ending in more-or-less severe chronic cardiomyopathy, depending on the degree of cardiac damage. Echocardiographic changes, which were observed in 21 of 40 pediatric patients after high-dose cyclophosphamide treatment (20), occurred approximately 1 week after treatment and persisted for days to weeks; pericardial effusion seen in 15 of these patients was successfully treated in 13, demonstrating the reversibility of these changes with careful management. Our data in the rat also demonstrate the reversibility of certain of the compositional changes in the heart after long periods of time, with the decrease in hydroxyproline content after Day 196 being of particular interest.

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Experimental Ultrastructural and X-ray Microanalysis Study of Cisplatin in the Rat: Intracellular Localization of Platinum[1,2]

J. P. Berry,[3,*] P. Brille,[4] A. F. LeRoy,[5] Y. Gouveia,[4] P. Ribaud,[4] P. Galle,[3] and G. Mathé[4]

Through the use of microanalytic techniques, it has been possible to demonstrate the presence of numerous elements in thin sections of tissue (1–3).

Cisplatin has been shown to be an effective therapeutic agent in the treatment of a certain number of types of cancers (4–7), but its use has been limited by its long-term toxicity in a number of organs including kidney, bone marrow, and liver (8–10).

Using methods of microanalysis, we have undertaken to establish the role of this element in the toxic effects manifested in the cells of different organs, ie, kidney, bone marrow (as a model of organ toxicity), and liver (as a test of platinum phagocytosis).

MATERIALS AND METHODS

Experimental Protocols

Fifteen 6-week-old male rats (Fischer 388) were treated with a weekly iv dose of 2.5 mg/kg of cisplatin (Cis-Platyl, Laboratoire Roger Bellon). The experiment was continued for 6 weeks. Controls consisted of 15 rats with the same characteristics that received weekly doses of physiologic saline solution. A similar study was conducted at a dose level of 3 mg/kg administered iv weekly.

Sample Collection

Samples of kidney, liver, and bone marrow were collected, in that order, from anesthetized rats (two treated, two controls) each week on the third day after each iv drug administration. The samples were fixed in a 1.5% glutaraldehyde solution in a pH 7.3 cacodylate buffer; some were also postfixed in a 1% solution of osmic acid in the same buffer. The samples were then washed in the cacodylate buffer, dehydrated, and mounted in Epon. Ultrathin sections of approximately 100 nm were cut using an LKB Ultro-tome III and mounted on aluminum or copper grids. None of the sections were stained.

Instrumental Methods

A Philips EM-300 electron microscope was used to study the ultrastructural morphology of the sections. A Cameca MBX electron microprobe was used to reveal the presence of the individual elements in the structure within the thin sections. The latter instrument is equipped with two wavelength-dispersive spectrometers. One is mounted inclined and is fitted with the following crystals: thallium acid phthalate (TAP), lead octadecanoate (ODPb), pentaerythritol (PET), and lithium fluoride (LiF). The vertically mounted spectrometer has PET and LiF crystals. The transmission electron microscope accessory on the electron microprobe magnifies on the order of 50,000 ×.

An ASR 33 Teletype unit is used to record X-ray signals automatically for preset counting times.

The experimental conditions used in these analyses were the following: accelerating voltage = 30 KV, beam current = 50 nA, and electron probe diameter = 500 nm.

The L_α X-rays of platinum were measured using the LiF crystal in the spectrometer, while the K_α X-rays of sulfur and iron were measured by means of the PET crystal. The X-ray counting for platinum was performed at its X-ray wavelength and background counting was accomplished at angles $\Theta + \Delta\Theta$ (0.33°). Each count was performed for 100 seconds and was repeated three times.

The statistical error in the measurement of the characteristic X-ray signal is given by $P_c = P - B$, were P = peak count signal and B = background count, and is estimated as $\sigma = \sqrt{P + B}$. The confidence interval is estimated from $P_c \pm 2\sqrt{P + B}$.

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[1] Received Sept 11, 1981; revised Mar 29, 1982; accepted Apr 13, 1982.

[2] Supported by the Institut National de la Santé et de la Recherche Médicale (INSERM) (France) under research contract 803021.

[3] Laboratoire de Biophysique-Centre de Microanalyse, S. C. R. 7 INSERM, Faculté de Médecine, Créteil, France.

[4] Institut de Cancérologie et Immunogénétique, INSERM U 50-Hôpital Paul Brousse, Villejuif, France.

[5] Biomedical Engineering and Instrumentation Branch, Division of Research Services, National Institutes of Health, Bethesda, MD.

*_Reprint requests to:_ Dr. J. P. Berry, Laboratoire de Biophysique-Centre de Microanalyse, S. C. R. 7 INSERM, Faculté de Médecine, 94000 Créteil, France.

The Student-Fisher test (t tables) (11) was used in establishing the significance of differences between the elemental signal and the background signal, based on the value of t, where

$$t = \frac{m_P + m_B}{\sqrt{\frac{s^2}{n_P} + \frac{s^2}{n_B}}}$$

and m_P = mean total count under the peak for each lysosome examined

m_B = mean background count

s^2 = standard deviation of the count (the square of the variance)

n_P = total number of countings made (three times the number of lysosomes examined)

n_B = n_p = number of background counts

and

$$s^2 = \frac{(x - m_P)^2 + (x - m_B)^2}{(n_P + n_B - 2)}$$

where x = total number of counts obtained in the preset time interval (100 seconds), m_P = average of three 100-second counts, ie, $x/3 = m_P$, and DF (degrees of freedom) = $(n_P + n_B - 2)$.

If | t | is less than the value given in the table of t values for the DF at the 95% confidence level, the difference is not significant. If | t | is greater than the tabulated value, the difference is significant, and the probability listed in the table for a given value of | t | indicates the degree of significance.

RESULTS

In all cases, the treated rats were found to lose weight progressively with successive doses of cisplatin. Inasmuch as the results obtained at both dose levels were essentially identical in all important regards, we report only those for the lower weekly dose.

Kidney

Electron microscopy: tubular lesions.—The major changes were in the proximal convoluted tubules. They were characterized by cellular necrosis (fig 1), which engendered progressive disappearance of the contents of the cell, by the appearance of lipid inclusions in the cytoplasm, and by the presence of accumulations of dense granules in the lysosomes. These alterations all developed progressively with time.

Dense granules in the lysosomes.—Small dense granules were found in all of the samples containing lysosomes and phagolysosomes. Some of the lysosomes contained clusters of large dense granules. With the sample taken the third week (total cisplatin = 10 mg/kg), these lysosomes seemed to open directly into vacuoles in the cytoplasm. With time, the clusters of granules in the lysosomes and the number of the lysosomes increased.

Electron microscopy: glomerular lesions.—After 1 month (total cisplatin = 12.5 mg/kg), several lesions appeared in the glomerular cellular epithelium that were characterized by the presence of microvilli and a dense material (believed to consist of protein in the intercytoplasmic vacuoles) (12).

Microanalysis.—Ten series of counts were made on each lysosome containing a cluster of very dense granules (fig 3). These showed a statistically significant difference between peak count and measured background levels. The results in table 1 thus show the presence of platinum in the lysosomes of the renal tubular cells only. Microanalysis (wavelength-dispersive spectrometry) also showed the presence of the elements sulfur and iron in the same intralysosomal structures (fig 2). The wavelengths of the respective peaks for each element are such that they are easily resolved.

Bone Marrow

Electron microscopy.—Comparison of samples from treated animals with those from controls showed a break in the continuity of the adipocytes and an apparent diminution of the erythrocyte precursors.

Microanalysis.—Demonstration of the presence of iron and sulfur was attempted on reticulocytes in both the treated animals and controls. No distinction could be made between samples.

Liver

In the sections of liver studied, neither electron microscopy nor electron probe microanalysis revealed any perceptible change when samples from treated rats were compared with samples from controls.

TABLE 1.—Statistical result of counting: X-ray microanalysis*

Cisplatin dose (mg/kg)	P_e	s^2	t
20	70.97 ± 41.39	2208.44	4.09
25	84.7 ± 46.34	1905.5	4.35

*P_e = statistical error in measurement of characteristic X-ray signal, s^2 = standard deviation of count, and t = Student's t test variable. t-Table probability (3.92; DF = 18) of $\alpha = 0.001$ for each dose.

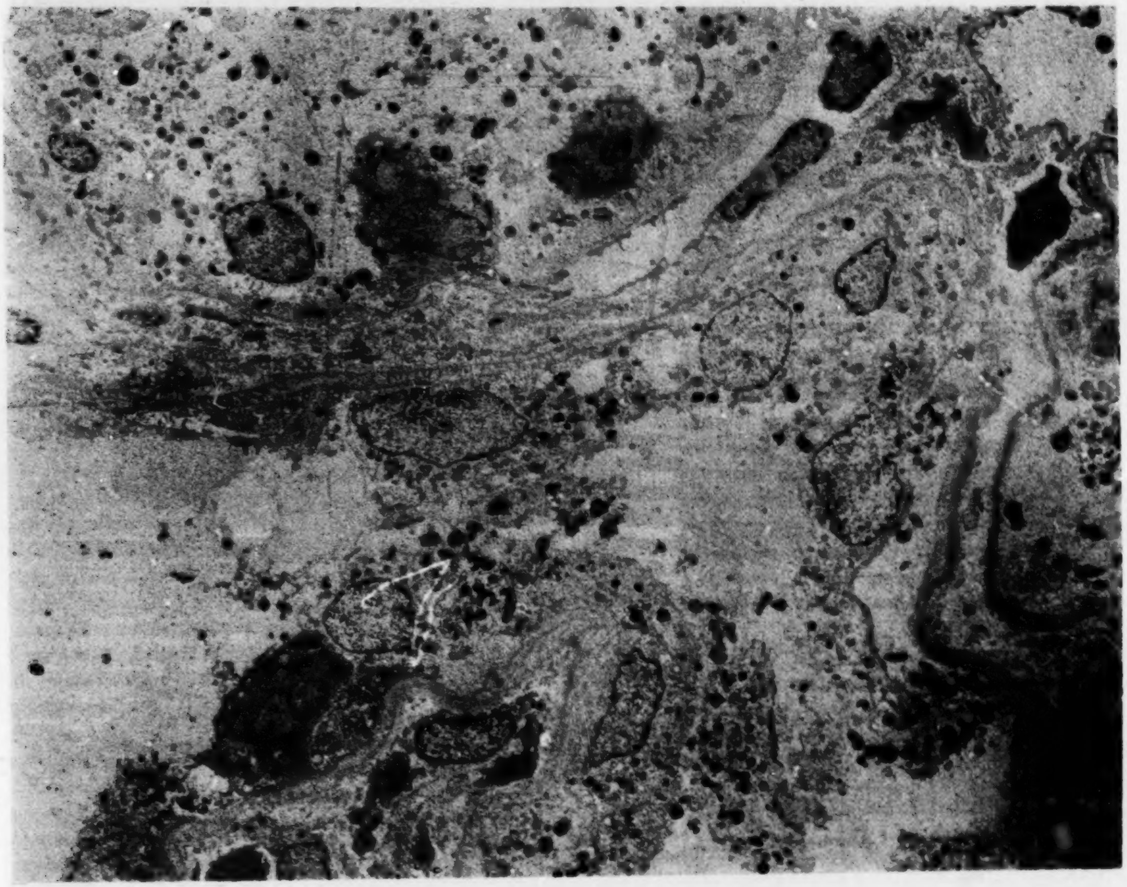

FIGURE 1.—Overview of tubular lesions in kidney of rat given 25 mg/kg of total cisplatin. Marked tubular necrosis characterized by a loss of cytoplasm. Remaining tissue is fragmented. × 3500.

FIGURE 2.—Proximal convoluted tubule in kidney of rat given 25 mg/kg of total cisplatin. Intracytoplasmic lysosome containing dense granulations. Microanalysis showed sulfur and iron. Counts demonstrated statistically significant presence of platinum. Upper right = brush border. × 12,500.

DISCUSSION

The experimental conditions used in this study set it apart from earlier animal studies in the following respects: (*a*) use of iv injections of cisplatin in the rats (simulating the mode of administration used with man) and (*b*) use of electron microscopy and microanalysis to study chronic toxic effects.

The results obtained are similar to those obtained in other studies of metal intoxication. The metals chromium, uranium, aluminum, and gold also concentrate in the lysosomes of kidney tubules (1–3). The metallic elements may be present alone (eg, chromium) (3) or may be associated with certain other elements such as phosphorus (eg, uranium and aluminum) (1,2). Further, uranium and aluminum precipitated as a phosphate in the presence of phosphoric acid and a lysosomal enzyme. In this experiment, the platinum is accompanied by sulfur, the same element that is associated with precipitates of gold (9) and mercury, as shown in previous microanalytic studies (13).

The nephrotoxic reactions to platinum are comparable to the effects of chronic chromium intoxication. Based on this comparison, this element could be re-released from the lysosomes as they spill their contents into the cytoplasm, which becomes necrotic and is carried off in the lumen (of the tubular cells) (3).

As the experiment progressed, the platinum was present in the lysosomes and may have been released in the cytoplasm.

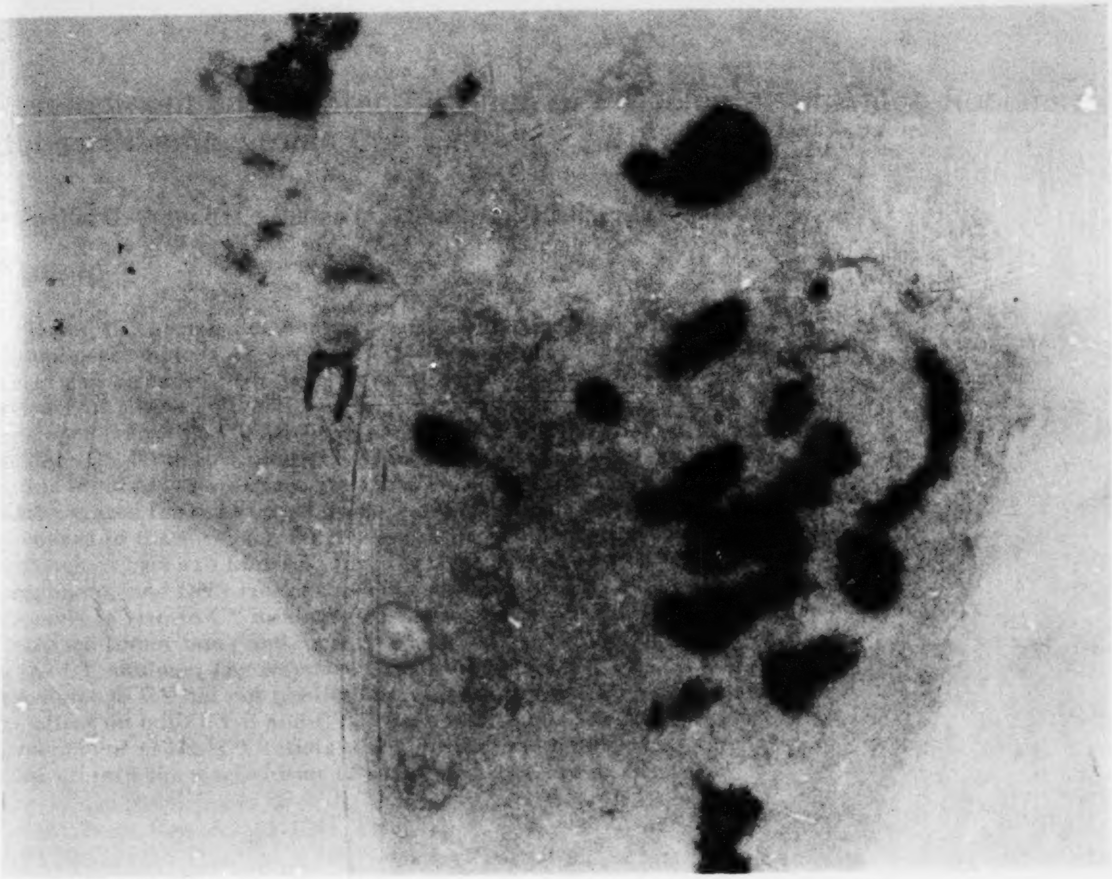

FIGURE 3.—Higher magnification (× 65,000) of fig 3, showing dense granules in lysosomal structure.

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Development and Characterization of a Cyclophosphamide-Resistant Mouse Plasmacytoma Cell Line[1,2]

Carmelita G. Frondoza,* Sudhir M. Trivedi, and Richard L. Humphrey[3,4]

SUMMARY

Murine LPC-1 plasmacytoma is sensitive to treatment with cyclophosphamide (CY), and animals bearing this tumor in advanced stages can be cured with doses as small as 60 mg/kg. With repeated transfer of LPC-1 cells followed by subcurative CY therapy, we have developed from this CY-sensitive parent line (CY-S) a stable CY-resistant subline (CY-R) that is unaffected by doses as large as 250 mg/kg. Stability of this induced CY resistance in the absence of CY treatment has been demonstrated for > 14 transfer generations. The CY-R subline has been compared to the CY-S parent line and shown to be similar with respect to morphology, growth kinetics, survival time, synthesis of IgG 2aκ M component, and DNA content and distribution. The CY-S parent line responds in vivo to CY analogs (ifosfamide and trofosfamide), as judged by decreased tumor mass and increased survival; the CY-R subline is resistant to the CY analogs. The response to carmustine (BCNU) was tested, and resistance to CY did not predict for response to this alkylating agent since the effect on both CY-S and CY-R lines was equal. The significance of the development of this CY-resistant subline and the implications for future research into the mechanisms of such resistance are discussed.

[Cancer Treat Rep 66:1535–1544, 1982]

Cyclophosphamide (CY) is a potent drug used in the treatment of plasma cell tumors (multiple myeloma) (1,2). However, patients undergoing prolonged CY chemotherapy often relapse, and subsequent treatment with this alkylating agent is no longer effective. This is a major problem in clinical oncology and severely limits successful palliation over extended periods of time. Such a pattern of acquired drug resistance has been attributed to the emergence of tumor cells that differ in their ability to evade or overcome the lethal effects of the drug. Alterations in cellular and molecular properties which may account for the development of resistance to alkylating agents have not been identified, although some biochemical differences between drug-sensitive and -resistant tumor lines such as small changes in thiol content and differences in cell permeability (ie, drug transport) have been observed (3–6). There is, therefore, a need for animal models that will allow additional studies of drug resistance at the cellular and molecular level. Knowledge of the biochemical mechanisms involved might serve as the basis for designing chemotherapeutic regimens that would inhibit transformation of drug-sensitive to -resistant tumors, avoid selection and amplification of drug-resistant cells, or restore resistant tumors to their original drug-sensitive state. Animal models could also provide tools for correlating and predicting by in vitro studies the in vivo response to alkylating agents. To date, only a few resistant sub-

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[1] Received Oct 13, 1981; revised Mar 9, 1982; accepted Mar 17, 1982.

[2] Supported in part by Public Health Service grants CA-06973 and CA-24698 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

[4] We thank Ms. Alverta Fields for excellent secretarial assistance.

**Reprint requests to:* Carmelita G. Frondoza, PhD, Oncology Center, The Johns Hopkins University School of Medicine, 600 N Wolfe St, Baltimore, MD 21205.

lines derived from animal tumors sensitive to alkylating agents have been developed, and no existing mouse plasmacytoma resistant to CY has been described (4–8). We have developed a murine CY-resistant (CY-R) subline from the CY-sensitive (CY-S) parent LPC-1 plasmacytoma, thus providing an animal model (particularly for multiple myeloma) that will allow analysis of changes in tumor cell properties following acquisition of drug resistance.

MATERIALS AND METHODS

Animals.—Female BALB/c mice weighing 20–25 g were obtained from Microbiological Associates, Bethesda, MD. They were given standard Charles River chow (RMH 1000) and water ad libitum.

Tumors.—LPC-1 CY-S plasmacytoma originally isolated by Dr. Michael Potter (National Institutes of Health, Bethesda, MD) was obtained from Litton Bionetics, (Rockville, MD). This tumor line was chosen as the myeloma model because of its consistent growth pattern in BALB/c mice and its production of a tumor-specific biomarker, IgG 2aκ M component. The secreted M component can easily be detected and quantitated by conventional electrophoretic methods and by more recent in vivo radiolabeling techniques (9). Tumor tissue was excised and cut into small pieces in RPMI-1640 tissue culture medium with 10% fetal calf serum (Microbiological Associates). The tissue fragments were further minced with a scalpel blade and passed through sterile gauze pads. Single-cell suspensions contained 0.2% trypan blue dye (0.2% dye in 0.9% NaCl solution). Cells were counted in a hemacytometer, and the volumes adjusted to 10^6 trypan blue-excluding cells/ml of medium. Cell morphology was examined with Jenner-Giemsa stain. The cells were maintained by serial sc or ip transfer of 10^6 cells every 3 weeks in 9-week-old female BALB/c mice.

Induction of CY resistance.—To induce a CY-R subline, mice were injected sc with 10^6 CY-S cells. Fourteen days later, mice bearing established tumors (~ 1 g) were given a subcurative dose of CY (30 mg/kg) ip. The tumors were allowed to regrow, and tumor cells were transferred 2 weeks later into another group of mice. Subsequent transfer generations were treated ip with gradually increasing CY concentrations (from 30 to 250 mg/kg). Development of resistance was followed by testing each transfer generation of cells in a separate group of ten mice with a curative ip dose of CY (125 mg/kg) on Day 14 after sc transfer of 10^6 cells.

Chemical reagents.—CY was obtained from Mead Johnson & Co (Evansville, IN). Trofosfamide, ifosfamide, and carmustine (BCNU) were donated by the Drug Synthesis and Chemistry Branch of the National Cancer Institute.

Isotopes.—6-^{14}C-arginine, 25.9 millicuries/mmol in 0.1 N HCl, obtained from New England Nuclear Corp (Boston, MA), was neutralized with 0.1 N NaOH and then diluted with an appropriate volume of 0.9% NaCl solution. Labeled amino acid (40 microcuries/mouse) was administered ip. ^{14}C-leucine, 339 millicuries/mmol in 2% ethanol, was also obtained from New England Nuclear Corp.

Collection of blood samples.—Blood was collected from the retro-orbital plexus using sterilized heparinized micropipets 24 hours following isotope administration. The micropipets were centrifuged, and the plasma obtained was frozen at -20°C until used.

Electrophoresis and radioautography.—Plasma (1 µl) was electrophoresed on Pol-E film (1% agarose/5% sucrose in 0.075 M barbital buffer, pH 8.6; Pfizer Diagnostics Division, Clifton, NJ) at 100 V for 50 minutes. The films then were soaked in 5% trichloroacetic acid containing 0.2% Ponceau S solution for 20 minutes. Excess stain was removed by washing with 5% acetic acid. The films were dried at 72°C and radioautographed using Kodak SB54 negatives for up to 4 weeks. The negatives were developed with a Model M6A-N Kodak RP X-OMAT processor. The electrophoretograms and radiograms were scanned at 525 nm with a Model 415 Clifford densitometer. The relative distribution of protein components (ie, albumin, γ-globulins, and M component) and radioactivity were determined based on the densitometric tracings. The M component region for each electrophoretic run was defined by unlabeled purified LPC-1 M component, placed in top and bottom wells of each membrane, and used as guide strips. This permitted the arbitrary assignment of radioactivity (from the radioautograms) and protein concentration (from the Ponceau S-stained membranes) to the M component, even when the M component concentration or radioactivity was too low to reveal a distinct homogeneous band.

In vitro Ig synthesis and secretion.—Viable LPC-1 cells were separated from other cells by one-step Ficoll-Hypaque gradient centrifugation (10). Tumor cell suspension (10^6 viable cells/ml) was prepared aseptically in leucine-free Eagle's minimum essential medium containing 25 mM Hepes buffer and 5000 units of penicillin-streptomycin (Microbiological Associates). Ten milliliters of the cell preparation in suspension tissue culture flasks (Bellco, NJ) was incubated with 20 microcuries of ^{14}C-leucine at 37°C. One-milliliter aliquots were centrifuged at 1000 rpm for 10 minutes, and the supernatant was obtained for electrophoresis and radioautography as well as for analysis of total and protein radioactivity. The cell pellet was resuspended in 1 ml of medium, and aliquots were cytocentrifuged for morphologic studies or lysed by repeated freeze-thawing to determine intracellular protein radioactivity.

DNA distribution analysis by fluorescence-activated cell sorting (FACS II).—Cells were stained with propidium iodide solution containing RNase and NP40 detergent according to the procedure of Vindelov (11). The nuclear preparation was then filtered through a 30-μ Millipore filter (13-mm diameter) and was analyzed in a FACS II (Becton and Dickinson, Los Angeles, CA). Details of instrumentation have been previously described (12). The fluorescent signals were displayed as a frequency distribution histogram on a 250-channel pulse-height analyzer. For standardization, chicken rbcs (CRBC) prepared by the method of Herzenberg and Herzenberg (12) or purified normal mouse spleen lymphocytes were added to the tumor cells. The coefficient of variation ranged from 3% to 5%.

RESULTS

Development of Mouse LPC-1 CY-R Subline

The CY-R subline of the LPC-1 plasmacytoma was developed by exposure of several tumor transfer generations to palliative (subcurative) doses of CY (30 mg/kg). In this mouse model, any host variability effects by previous CY exposure were eliminated by rescuing the treated and recovered tumor cells and transferring a standard tumor cell dose (1 × 10^6 cells) into a fresh host. Resistance to CY was monitored during the development of this CY-R line by challenging a separate group of mice bearing sc tumor of that generation with a curative dose of drug (125 mg/kg). A prolonged period of drug exposure with as many as nine tumor passage generations was required for resistance to be detectable, and up to 18 generations were required before CY resistance was complete (fig 1). After the ninth tumor cell transfer, development of drug resistance

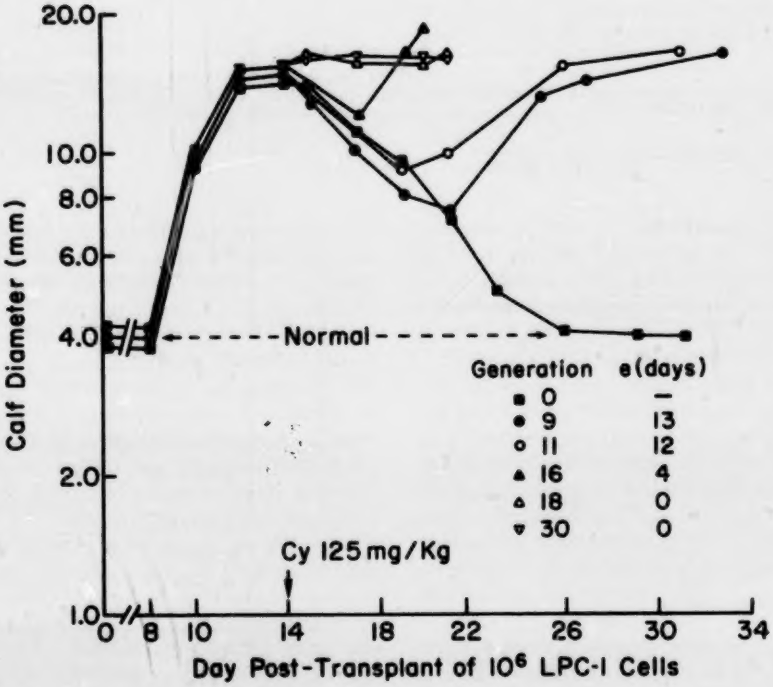

FIGURE 1.—Assay for CY resistance. Response to CY as a function of generation time. A group of 10 mice received a "curative" dose of CY ip (125 mg/kg) 14 days after sc cell transfer of 10^6 cells. Calf diameters (mm) were the mean of 2 caliper measurements taken at right angles to each other. 5 representative generations of tumor growth are shown. "e" (days) = time required for the tumor to resume pretreatment size.

TABLE 1.—Development of CY resistance

Generation No.	Median survival time (days) of mice	
	Untreated	Treated*
0†	25	> 60‡
9	25	34
18	25	25
24	25	25
35	25	25

*125 mg/kg of CY.
†Parent, CY-S.
‡"Cured."

was indicated by a transient regression in tumor volume after challenge with a single curative dose of CY, followed by regrowth (fig 1). In contrast, the parent tumor, CY-S (generation 0), completely disappeared and did not recur. The time interval (e) required for the tumor to resume its pretreatment size was shorter with each transfer generation (fig 1). By passage 18, there was no significant decrease in tumor size following a drug dose which was curative for the parent, drug-sensitive tumor of similar size. The progressive development of drug resistance was further demonstrated by the shorter survival times (25 days) seen in the later generations (table 1, generation 18 and beyond) in contrast to the earlier generations (table 1, generation 9) which displayed a prolongation of survival (34 days) but not a "cure."

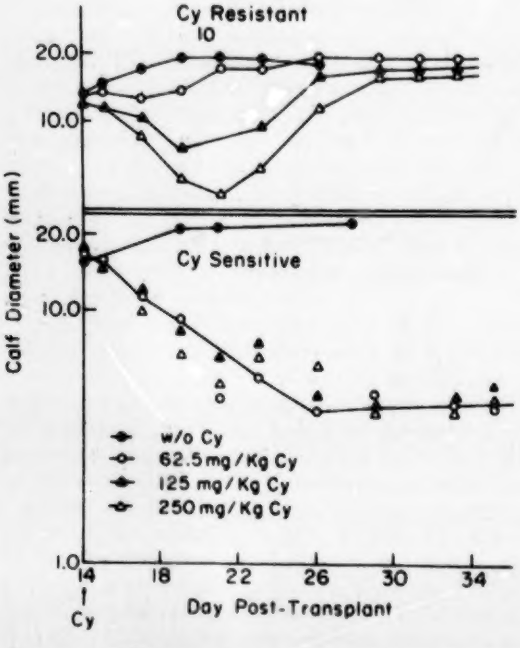

FIGURE 2.—LPC tumor growth with CY. Tumor regression (calf diameter) following curative CY treatment comparing CY-S parent line to CY-R_{10} line (generation 10).

Earlier generations in the development of CY resistance showed a dose-dependent response to CY (fig 2). When the lowest curative dose of CY (62.5 mg/kg) was administered, the least tumor regression and earliest recurrence were observed (fig 2). Subsequent CY-R passages (ie, by generation 18) no longer responded to these drug concentrations. Once CY resistance status was achieved, the tumor remained drug-resistant despite prolonged (> 14 generations) passage in the absence of CY exposure.

Characterization of CY-R Tumor Cells

Similar to their CY-S parent, CY-R cells induced tumors in their BALB/c syngeneic hosts with the same growth kinetics depending on the cell doses (fig 3). Both tumor lines (at cell doses of 1 × 10^6)

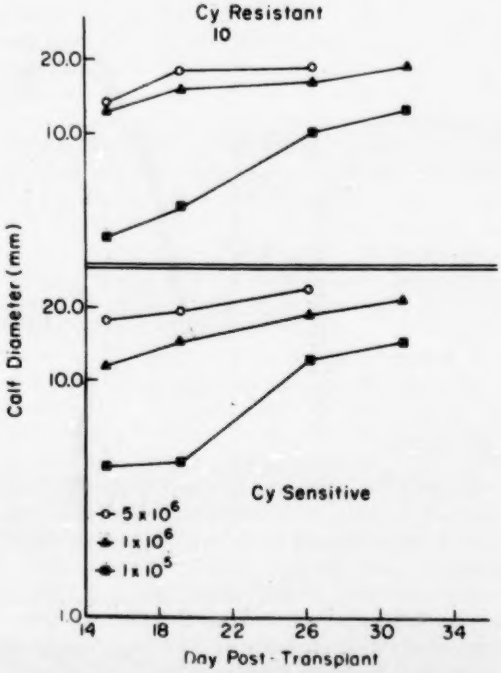

FIGURE 3.—Tumor growth curves indicated by increase in calf diameters from 10 mice following sc injection of various tumor cell numbers. CY-S parent line is shown in the bottom panel for comparison with CY-R_{10} (generation 10) subline in the top panel.

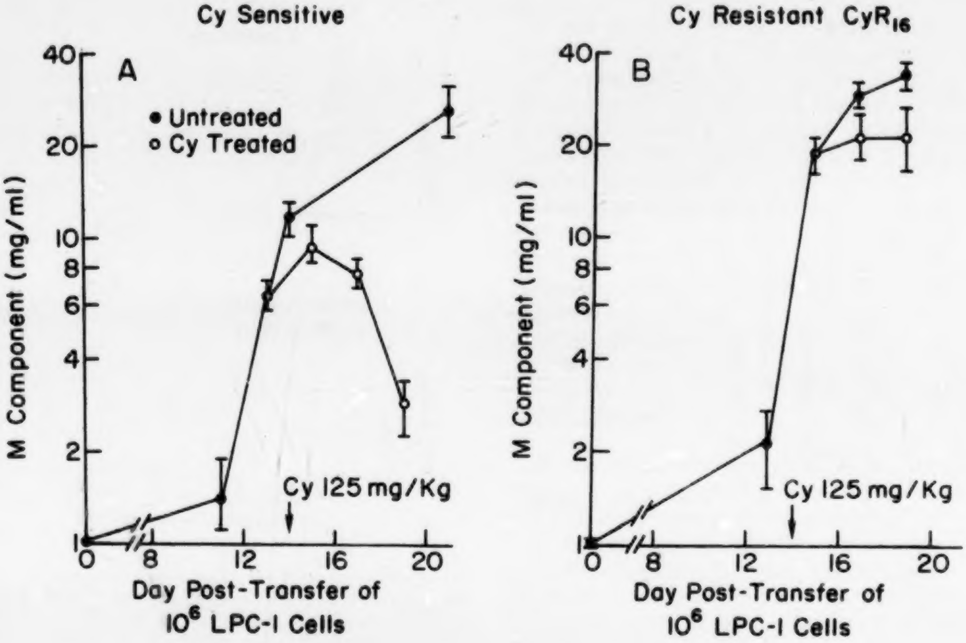

FIGURE 4.—Effect of CY on M component concentration. Tumor growth/regression curves indicated by M component concentration following sc injections of 10^6 tumor cells. This experiment was from generation 16 (CY-R_{16}), before CY resistance was fully developed. As shown in *B*, CY treatment had a temporary modulating effect on M component concentration.

killed mice in 4–5 weeks, with median survival times of 24–26 days. Tumor growth kinetics measured by the emergence and levels of the tumor M component (IgG 2aκ) were very similar in both (CY-S and CY-R) cell lines. The IgG 2aκ M components produced and secreted by CY-S and CY-R cells were identical in their electrophoretic mobility on agarose (9). Plasma M component concentration monitored in both CY-S and CY-R untreated groups of tumor-bearing mice showed the same time-dependent increase during the period of tumor growth (*see* control curves, fig 4). Tumor M component levels of CY-S tumor-bearing mice consistently decreased and were undetectable 2 weeks after CY treatment (125 mg/kg). In contrast, plasma M component concentrations of generation 16 CY-R tumor-bearing mice did not significantly decrease following drug administration (fig 4). The plateau observed after CY treatment was due to the transient regression of tumor in this early generation in the development of the CY-R trait, as shown above in figure 1. In vitro M component synthesis rates of the two cell lines were compared by measuring ^{14}C-leucine incorporation into tumor protein during short-term, 24-hour culture. There was similar time-dependent incorporation of the radiolabeled amino acid into M component, with similar in vitro synthesis rates of 22,000–46,000 molecules of IgG 2aκ/cell/minute.

From a morphologic viewpoint, CY-S and CY-R tumors were very similar in that both exhibited two cell types comprising the tumor: plasmacytoid (33%–52%) and lymphocytoid (48%–67%) and insignificant numbers of granulocytes or other mononuclear cells. Both plasmacytoid and lymphocytoid cell types contained intracellular IgG 2aκ but no detectable cell-surface Ig when examined by indirect immunofluorescence and Sternberger's unlabeled antibody-antiperoxidase technique (13).

The DNA distributions of CY-S and CY-R tumors were indistinguishable when analyzed by FACS II, which measures the intensity of cellular fluorescence proportional to DNA content (12). The standards used were CRBC and diploid normal mouse spleen lymphocytes which showed peak fluorescence at about channels 20 and 40, respectively (fig 5). Tumor cells stained with propidium iodide re-

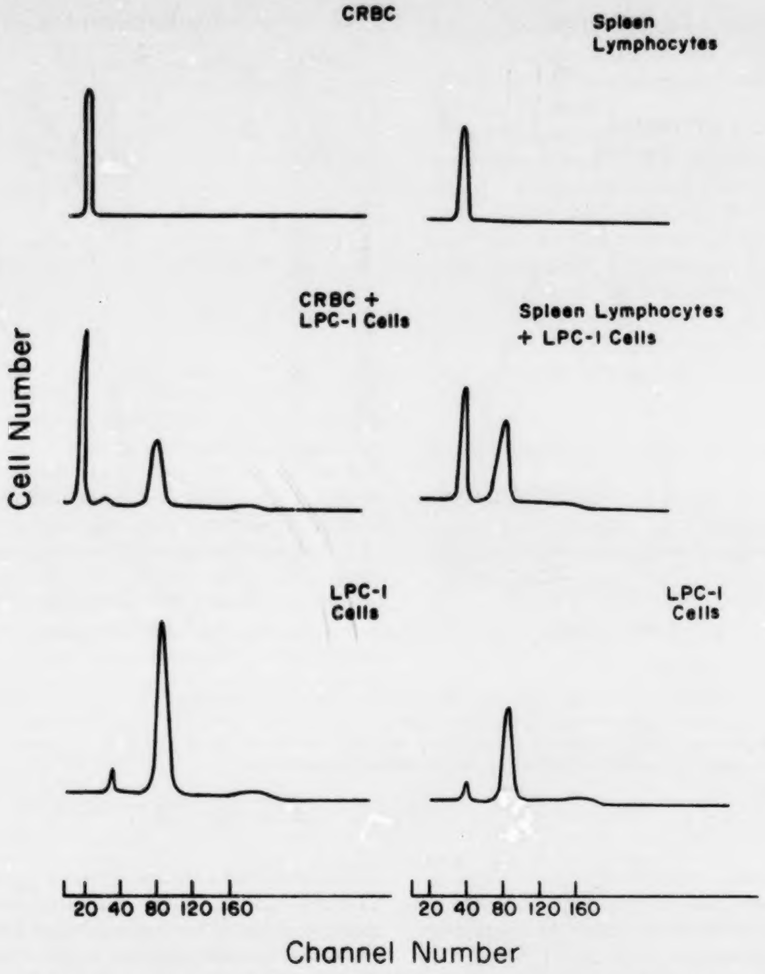

FIGURE 5.—DNA histogram. Representative experiments illustrating the DNA distribution of LPC-1 CY-S or CY-R plasmacytoma cells as determined by FACS II analysis of propidium-stained nuclei. CRBC served as the control for the experiment shown at the left, and normal mouse spleen lymphocytes served as the internal standard for the experiment shown at the right. The middle panel illustrates the results of cell-mixing experiments, and top and bottom panels show the unmixed control cells and the LPC-1 tumor cells, respectively.

vealed three discrete peaks of DNA fluorescence by FACS II analysis (fig 5). The first peak at about channel 40 represents diploid DNA based on normal mouse spleen lymphocytes as an internal control. The second and dominant peak, polyploid (approximately tetraploid), centered around channel 80, and the minor third peak was around channel 160.

To rule out the possibility that solid (sc) CY-R tumor was resistant due to nonspecific host factors affecting tumor growth, such as poor vascular supply, drug inaccessibility, or hypoxia, tumor cells were grown ip as ascites. CY doses curative for CY-S tumors failed to eliminate CY-R tumors grown either sc or in ascitic form. As shown by measurements of tumor M component, CY-R sc and ascites tumors continued to grow following CY treatment (fig 6). These CY-R tumor-bearing mice died by 4–6 weeks.

Experiments were also conducted to determine whether CY-R cells would be more sensitive to the

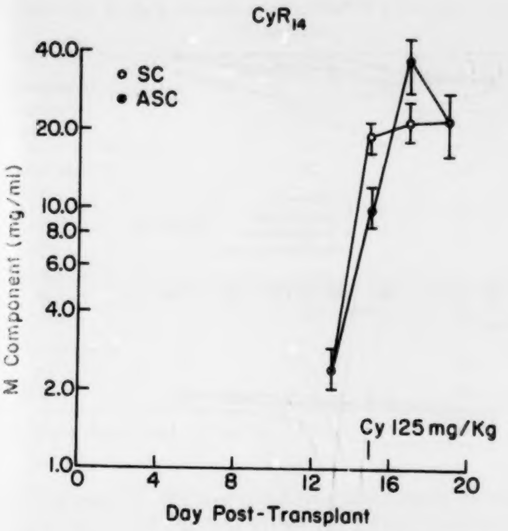

FIGURE 6.—2 groups of 10 mice each received 125 mg/kg of CY ip 14 days after ip and sc transfer of 1 × 10^6 CY-R_{14} cells (generation 14). Tumor IgG 2aκ M component mean values ± SEM are presented. sc = subcutaneously growing tumor; asc = ascitic tumor.

drug after cell transfer during the "eclipse" phase, the period when tumor cells have not yet been fully established (14). CY was administered following sc or ip transfer of cells, before tumor or ascites and M component were detectable (Days 0–7). CY-R cells continued to grow in mice, as shown by the subsequent increase in tumor size or the development of ascites and the rise in plasma tumor M component. Mice bearing CY-S tumors treated with drug during this time interval (ie, 0–7 days) did not develop detectable tumor ascites, or M component.

Cross-resistance of the CY-R tumors to other alkylating agents structurally similar to CY also was investigated. The response of CY-S or CY-R tumors to CY analogs (ifosfamide and trofosfamide) was tested. Ifosfamide treatment (62.5 or 125 mg/kg of body weight) resulted in regression of established sc CY-S cells and prolongation of survival times from 25 to 48 days (fig 7), whereas CY-R tumors did not respond to ifosfamide at all (fig 7). Trofosfamide induced complete regression of CY-S tumors and prolonged survival times > 60 days (cured) but induced only transient regression of CY-R tumors (fig 7).

Earlier studies indicated that other CY-resistant tumor lines (eg, L1210/CPA leukemia) were not cross-resistant to BCNU or to other functionally and structurally different alkylating agents (7). A similar lack of cross-resistance was observed when the sensitivity of our LPC-1 CY-R tumors to BCNU was investigated. Various doses of the drug were administered ip to mice bearing established sc CY-S or CY-R tumors. A dose of 6 mg/kg was toxic to these mice (20 of 20 died by Day 3), and nonlethal doses of 3, 1.5, and 0.7 mg/kg yielded a narrow dose-response curve as shown in figure 8. Both CY-S and CY-R tumors regressed after treatment with 3 mg/kg as indicated by the decrease in tumor mass (calf diameters) and prolongation of survival time > 60 days (cured; table 2). The lower doses neither reduced tumor mass nor increased survival times (fig 8).

DISCUSSION

In the studies presented here, we described and characterized the first mouse myeloma tumor line (LPC-1) to develop resistance to the alkylating agent CY. Similar to the induction of resistance to other alkylating agents in many murine tumors, development of CY resistance by the LPC-1 plasmacytoma was a slow process requiring progressive drug treatment for several transfer generations (5–8). Once resistance was achieved, the tumor grew unperturbed in the presence of curative doses of the drug. In addition, this CY-R subline has maintained its CY resistance for > 14 transfer generations in the absence of drug exposure. Earlier studies on L1210 leukemia cells showed that CY resistance in the absence of drug could be maintained for > 130 generations (7).

The gradual emergence of CY resistance suggests the gradual evolution of the tumor cell population toward CY resistance by means of the selection pressure of subcurative drug treatment operating on an original tumor cell population heterogeneous for the CY resistance trait. This kind of intrinsic tumor heterogeneity has been suggested by the observable range of variation among cells comprising the tumors, eg, pleomorphic morphology, heterogeneity of biomarker production, selective organ metastasis, and variations in drug sensitivity (1,2,4,5). Tumor heterogeneity with respect to drug sensitivity has been suggested in other cell lines by the emergence of alkylating agent-resistant tumor cells after prolonged low-dose treatment, although a few drug-resistant cell lines (eg, semustine-resistant Lewis lung carcinoma, B16 melanoma, and CY-resistant L1210 leukemia cells) have been obtained after single high-dose administration (7). Skipper and Schabel (3) also developed a CY-resistant Fortner hamster Pla. 1 plasmacytoma with a shorter period of drug exposure. In those studies, the rapid development of drug-resistant cells sug-

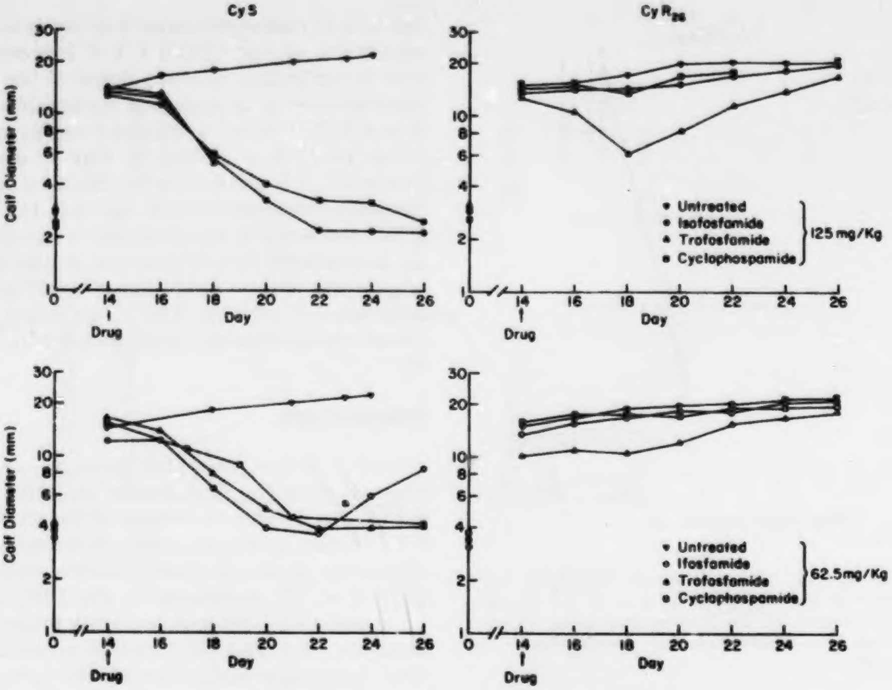

FIGURE 7.—Effect of CY analogs on LPC-1 tumor growth. Mice bearing established sc tumors were treated ip with CY or its analogs, and calf diameter measurements were taken at different time intervals; CY-R$_{26}$ is generation 26.

gests sufficiently high frequency of these cells within certain tumor populations, allowing immediate amplification during high-dose drug exposure.

Other than the difference in their sensitivity to CY, the parent LPC-1 CY-S and the CY-R tumor subline exhibit the same cellular properties with respect to (*a*) growth kinetics, (*b*) synthesis and secretion of tumor IgG 2aκ M component, (*c*) morphology, and (*d*) DNA distribution. These findings are in agreement with earlier studies such as those of Goldenberg (8) and Schabel et al (15) done on other tumor systems where drug-sensitive and -resistant cell lines exhibit similar growth rates and similar cytologic and biochemical features.

FACS II analysis indicated identical DNA distribution (fig 5) of the parent CY-S and CY-R tumor cells, suggesting similar ploidy. Previous studies also failed to demonstrate significant differences in the ploidy as well as the karyotype of alkylating agent-sensitive and -resistant tumor cells (8,16,17). Goldenberg (8) earlier reported increased percentage of tetraploid cells in association with mechlorethamine resistance, but considerable overlap was also noted between drug sensitivity and polyploidy. Chromosome analyses on our CY-R and CY-S lines have not yet been performed.

Other studies have demonstrated that structural differences or similarities in alkylating agents may predict for biologic effects and for cross-resistance. We have examined the ability of our CY-R tumor cell line to respond to closely related drugs (the CY analogs ifosfamide and trofosfamide), and another, structurally distinct, alkylating agent (BCNU). Ifosfamide differs from the parent compound in that its second chloroethyl group is attached to the ring nitrogen whereas trofosfamide has a third additional chloroethyl group in this position. The CY-R tumor was also resistant to these analogs (fig 7, table 2), indicating that changing the position and number of chloroethyl groups could not overcome the drug resistance at the doses used (62.5 and 125 mg/kg). It is interesting that trofosfamide treatment extended survival times of CY-R tumor-bearing mice more than CY did, since this analog has two additional chloroethyl groups.

When a structurally different alkylating agent (BCNU) was used, the CY-R tumor behaved like its CY-S parent; both tumors responded, and both

TABLE 2.—Response comparison of LPC-1 CY-S and CY-R to CY analogs

Treatment and dose mg/kg)	Median survival time (days) of mice bearing—	
	LPC-1 CY-S	LPC-1 CY-R
Untreated	25	25
Y		
62.5	> 60	25
125.0	> 60	25
fosfamide		
62.5	48	28
125.0	48	28
rofosfamide		
62.5	> 60	20
125.0	*	39

*All animals died by Day 18.

CY-S and CY-R tumor-bearing mice were cured (ie, survival > 60 days). However, the murine host (BALB/c mice) failed to tolerate higher drug doses, and lethal toxicity was observed at twice the curative dose. Thus, the dose-response curve analyzed was quite narrow (fig 8).

The BALB/c mouse LPC-1 plasmacytoma has been in continuous, serial in vivo passage for more than two decades. Over this period, it has exhibited a stable growth pattern and the continuous production of a tumor-specific IgG 2aκ M component (9,14,16). In the studies presented here, we report the development of a CY-R LPC-1 plasmacytoma which, by all criteria studied, is indistinguishable from the CY-S parent line. For the first time, a mouse myeloma model is available that will permit the analysis of cellular and molecular changes which could account for the development of tolerance of myeloma cells to alkylating agents. These special features of our model offer promising investigational approaches to the biology of the development of resistance to alkylating agents.

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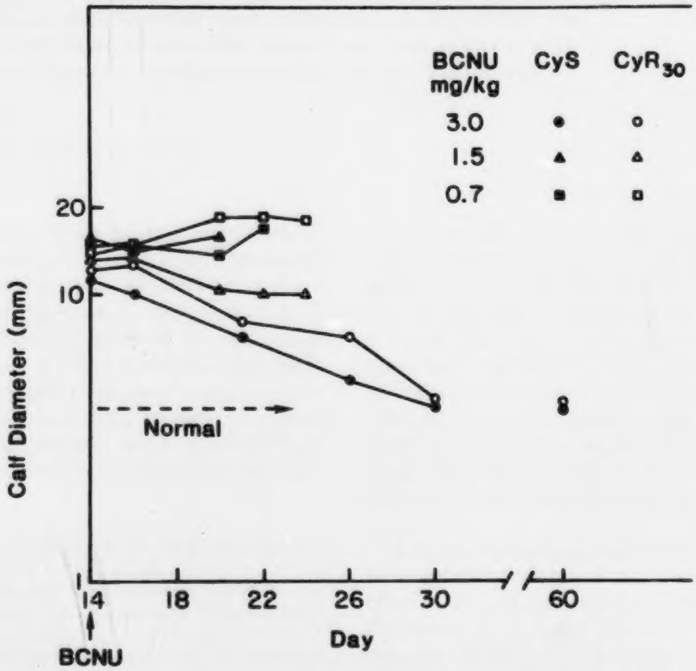

FIGURE 8.—Dose-response curve of LPC-1 plasmacytoma to BCNU. Mice bearing sc CY-S or CY-R tumors were injected ip with varying doses of BCNU, and calf diameters were monitored at different time intervals; CY-R_{30} is generation 30.

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Modification of Radiation Damage in Rat Spinal Cord by Mitotane[1,2]

Arvin S. Glicksman,* Sarah F. Bliven, and John T. Leith[3]

SUMMARY

Modification of the paralytic response in rats after 6-MV photon irradiation of the spinal cord with either single or split exposures (two equal fractions given in a 24-hour period) by mitotane was investigated. Mitotane was administered as a suspension in physiologic saline (300 mg/kg/day) for either 5 days prior to or 5 days after irradiation. For rats receiving split doses of 6-MV photons, either the last two doses of mitotane were given 2 hours prior to each radiation fraction or mitotane was begun 2 hours after the second fraction and continued for 5 days. The data to 6 months after irradiation indicate that, in rats given mitotane for 5 days *prior* to single-dose photon irradiation, the paralytic response (as defined by the dose needed to produce paralysis in 50% of the irradiated groups of rats) was enhanced by a dose-enhancement factor (DEF) of 1.40. The DEF in the group of rats given mitotane *after* single doses of 6-MV photons was 1.15. In the split-dose irradiation experiments, the DEF for the groups of rats given mitotane *prior to* each radiation fraction was 1.36; while the DEF for the group of rats receiving mitotane beginning *after* the second fraction was 1.18. These data indicate that mitotane can potentiate the effects of 6-MV photon irradiation to the central nervous system, with mitotane administered prior to irradiation being the most effective sequence.

[Cancer Treat Rep 66:1545–1547, 1982]

Mitotane (*O,P'*-DDD) is an isomer of the insecticide tetrachlorodiphenylethane (DDD). This agent has been used in the palliative treatment of inoperable adrenocortical carcinoma. Bergenstal et al (1) and Lubitz et al (2) have described the antitumor activity of this compound, and it is used in the treatment of metastatic disease (3,4) and Cushing's syndrome (5,6). Mitotane has been shown to induce severe adrenal cortical atrophy when given to dogs (7), an effect that appears to be mediated through effects on the mitochondria of the adrenal cortical cells, leading to focal lesions in the fascicular and reticular zones of the adrenal cortex (8). Concomitantly, secretion of steroids by the adrenal gland is reduced (9). The drug appears to be stored primarily in fatty tissues throughout the body (10) and can be detected in the blood for several months after administration is discontinued. While the liver appears to metabolize relatively small amounts of the drug, the bile appears to be a significant organ of elimination (as the ethene or acetate derivatives of mitotane).

A large number of patients treated with the drug have exhibited central nervous system (CNS) side effects, including lethargy, sedation, vertigo, and dizziness. Long-term use may lead to overt brain damage (2,10). As this agent has been shown to produce damage in the CNS, we wanted to determine whether it would interact with damage to the CNS produced by sparsely ionizing radiation. We irradiated the thoracolumbar regions of the verte-

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[1] Received Nov 4, 1981; revised Feb 17, 1982; accepted Feb 25, 1982.

[2] Supported by Public Health Service grants CA-80723 and CA-25687 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Radiation Biology Laboratories, Department of Radiation Oncology, Rhode Island Hospital, and Section on Radiation Medicine, Brown University, Providence.

**Reprint requests to:* Arvin S. Glicksman, MD, Department of Radiation Oncology, Rhode Island Hospital, 593 Eddy St, Providence, RI 02902.

bral columns of rats with graded doses of either single or split (two equal fractions) doses of 6-MV photons. This model for radiation-induced myelopathy has been used by us for investigation of high linear energy transfer (LET) radiation on the spinal cord (11) and for investigation of the potentiation of low LET ionizing radiation on the spinal cord by whole-body hyperthermia (12). The theoretic implications of data derived from this normal tissue model system have been published (13).

MATERIALS AND METHODS

The rats used were adult CDF females obtained from the Charles River Breeding Laboratories, Wilmington, MA. They were housed in large, wire, hanging cages and were given food (Purina Lab Chow, Fort Wayne, IN) and water ad libitum. Rats weighed about 180 g at the time of treatments.

For irradiation, rats were exposed with a 6-MV Siemanns linear accelerator. Rats were anesthetized with sodium pentobarbital ip (50 mg/kg) and were exposed transversely to the thoracolumbar (T12-L1) region of the vertebral column. A source-to-skin distance of 50 cm was used, with the dose calculated at the center of the vertebral column. The field was collimated so that a 2-cm length of vertebral column was exposed, and a bolus of beeswax was placed over the dorsal region of the rat so that the incident photon beam encountered a constant amount of material before reaching the position of the spinal cord. The dose rate was about 5 Gy/minute.

The mitotane (Bristol Laboratories, Syracuse, NY) was prepared from tablet form. Administration of this agent in humans is by mouth, over a long period of time at relatively high doses. The tablets (500 mg each) were initially crushed to a fine powder using mortar and pestle. Each tablet was suspended in 16.6 ml of physiologic saline to give a drug concentration of 30 mg/ml. The rats were given 300 mg/kg of mitotane (about 1.8 ml of suspension) per administration. This drug suspension was administered daily for 5 days by stomach tube to rats that had been lightly anesthetized by methoxyflurane (Metofane; Pitman-Moore, Inc, Washington Crossing, NJ). To check on the reproducibility of our administration, replicate samples of drug suspension were withdrawn from the stirred solution and evaporated to dryness; the remaining powder was weighed. It was determined that each dose administered to the rats was accurate to within 1.03% (95% confidence limits).

For the combined photon irradiation and drug experiments, the protocol shown in table 1 was used. There were five dose levels (including no irradiation for controls) used per treatment regimen, with eight rats per dose level. These combined treatments were given in two sequences (ie, drug either before or after irradiation) and with two dose schedules (single and split doses of irradiation) and were intended to determine whether mitotane had any potentiating effect on spinal cord damage, what the effect of sequencing was, and whether the drug had any effect on repair of subeffective radiation injury in the spinal cord.

RESULTS AND DISCUSSION

We determined the dose needed to effect paralysis in 50% of the irradiated groups of rats, with and without mitotane administration. The endpoint chosen was equivalent to induction of a definite but mild impairment of hindlimb kick, extensor and flexor

TABLE 1.—Treatment schedule*

	Day of treatment										
Treatment	1	2	3	4	5	6	7	8	9	10	11
Single doses											
R alone						R					
M + R	M	M	M	M	M	R					
R + M						R	M	M	M	M	M
Split doses											
R alone						R_1	R_2				
M + R		M	M	M	M	M + R_1	M + R_2				
R + M					R_1	R_2 + M	M	M	M	M	M

*R = radiation treatment. Single-dose radiation levels were 21.25, 23.75, 26.25, and 28.75 Gy. R_1 and R_2 denote equal split-dose radiation treatments. Split exposure total doses were 29.50, 32.50, 35.50, and 38.50 Gy. Mitotane (M) was administered at a dose of 300 mg/kg. In M + R_1 and M + R_2 treatments, mitotane was given 2 hrs prior to each radiation factor; in R_2 + M treatment, mitotane was given 2 hrs after second radiation treatment.

TABLE 2.—Estimated ED50 and DEFs after spinal cord irradiation in the rat

Treatment*	ED50 (Gy)†	DEF‡
Single doses		
R alone	28.50 ± 1.5	—
M + R	20.50 ± 1.3	1.39 ± 0.11
R + M	24.50 ± 1.2	1.16 ± 0.08
Split doses		
R alone	35.50 ± 2.1	—
M + R	26.00 ± 1.8	1.36 ± 0.12
R + M	30.00 ± 2.0	1.18 ± 0.10

*R = radiation treatment (6-MV photons); M = mitotane.

†Values = mean ± SE (95% confidence limits).

‡Values = DEF ± SE. Errors are propagated 95% confidence limits.

activities, based on a subjective grading scale that we have used in other studies (11). These are early data, up to 6 months after irradiation. To date, we have not lost any animals in our study.

The doses of radiation needed to produce 50% myelopathy in rats as a function of treatment schedule are shown in table 2; the dose-enhancement factors (DEFs) found by comparing these ED50 values are also listed. Error limits on the ED50 and DEF values were calculated by methods previously described (11). For both the single and split photon irradiation schedules, mitotane given prior to irradiation yields DEFs of 1.36–1.39; for mitotane given after photon irradiation, the DEFs are 1.16–1.18. Therefore, mitotane is more effective in potentiating photon injury to the spinal cord when present prior to irradiation. Also, if the fractional amount of radiation damage repaired is calculated (11), the repair values are similar for all situations (ie, photon irradiation alone or photon irradiation plus mitotane given either prior to or after irradiation). This suggests that mitotane is acting as a pure sensitizer and is not acting through inhibition of the repair of subeffective radiation damage in the spinal cord.

Mitotane has been used periodically in the management of adrenal cortical carcinoma, particularly when it has been associated with endocrine-secreting syndromes (14). It has been effective in many of these situations without significantly affecting tumor control or survival (15). The drug also has a role in the management of Cushing's syndrome and may be used in patients who have previously been treated with radiotherapy. These early data indicate that mitotane does interact with damage to the CNS produced by sparsely ionizing radiation and enhances the radiation effect. Therefore, the inadvertent combined use of the drug and radiation may result in an unexpected CNS injury, which neither the drug alone nor the radiotherapy alone would have produced. On the other hand, utilization of this sensitization effect for potential therapeutic gain is a possibility that needs further investigation.

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Treatment of Neoplastic Meningitis With Intraventricular Thiotepa and Methotrexate[1,2,3]

Brief Reports

Donald L. Trump,* Stuart A. Grossman, George Thompson, Kenneth Murray, and Moody Wharam[4]

SUMMARY

Twenty-five patients with involvement of the meninges by disseminated cancers were treated weekly with intraventricular thiotepa (10 mg) and methotrexate (10 mg) and with radiation therapy. Twenty-two of the 25 patients were clinically improved or stable. Malignant cells were cleared from the cerebrospinal fluid in 76% of the patients. Myelosuppression was the dose-limiting toxic effect, and neurotoxicity occurred in three patients. Combination chemotherapy employing thiotepa and methotrexate is a safe and effective treatment for neoplastic meningitis.

[Cancer Treat Rep 66:1549–1551, 1982]

Increased attention has been focused recently on leptomeningeal involvement by disseminated cancer (neoplastic meningitis) (1–4). The prognosis for such patients is poor, with a median survival of 1½–4 months (5,6). Recommended treatment for neoplastic meningitis includes radiation therapy to symptomatic regions of the neuroaxis and intrathecal cytotoxic chemotherapy given through an intraventricular catheter (7).

Improved treatment of neoplastic meningitis may be attained by developing new intrathecal drugs and drug combinations. This report describes a phase II evaluation of combination intrathecal chemotherapy employing thiotepa and methotrexate.

MATERIALS AND METHODS

Twenty-five patients were treated for neoplastic meningitis. Twenty-three patients had a positive cerebrospinal fluid (CSF) cytology, and two patients had disseminated cancer, multifocal neurologic defects, parameningeal foci of cancer, and an abnormal CSF (table 1). Panmyelography and cerebral computer-assisted tomographic scans were performed in each patient.

All patients received radiation therapy (2500–3000 rad/10 days) to symptomatic areas of the neuroaxis. Intrathecal chemotherapy was begun within 7 days of the recognition of neoplastic meningitis and was administered through a subcutaneous reservoir and intraventricular catheter. Treatment consisted of the weekly administration of thiotepa (10 mg in 10 ml of buffered diluent on Day 1) and methotrexate (10 mg in 10 ml of buffered diluent on Day 4). Weekly therapy was continued for either 8 or 12 weeks, depending on the rapidity of CSF and clinical re-

TABLE 1.—Patient characteristics

Characteristic	No. of patients (%)
Total in study	25
Sex	
Female	18
Male	7
Median age in yrs: 50 (range, 22–76)	
Primary diagnosis	
Breast cancer	16 (64)
Lung cancer	2 (8)
Lymphoma/leukemia	4 (16)
Other cancers	3 (12)
Parenchymal brain metastasis	10 (40
Epidural spinal cord compression	10 (40)
Positive CSF cytology	
Lumbar	23 (92)
Ventricular	12 (48)
Active, progressive cancer outside the CNS	15 (60)

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[1] Received July 9, 1981; revised Feb 11, 1982; accepted Mar 4, 1982.

[2] Supported in part by Public Health Service grant CA-17970 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Dr. Trump is the recipient of an American Cancer Society Junior Faculty Clinical Fellowship.

[3] Presented in part at the Meeting of the American Association of Cancer Research, Washington, DC, April 1981.

[4] Johns Hopkins Oncology Center and Department of Neurosurgery, Johns Hopkins Medical Institutions, Baltimore, MD.

**Reprint requests to:* Dr. Donald L. Trump, Wisconsin Clinical Cancer Center, K4/662, CSC, 600 Highland Ave, Madison, WI 53792.

sponse. After induction therapy, responding patients were treated for 8 or 9 additional months, with 1 week of thiotepa and methotrexate monthly. If myelosuppression or stomatitis occurred, oral leucovorin factor was administered after intrathecal methotrexate. All 25 patients received at least 2 weeks of intrathecal therapy.

Required treatment for systemic cancer was administered concurrently with therapy for neoplastic meningitis. In 18 patients large doses of corticosteroids were administered for the first 14–35 days of treatment.

RESULTS

Clinical Response

Three patients (12%) experienced progressive deterioration in neurologic signs and symptoms during therapy. Thirteen patients (52%) were neurologically stable for at least 6 weeks following initiation of therapy. Five patients had definite partial improvement in neurologic signs and symptoms, and in four patients (16%) complete resolution of all neurologic signs and symptoms occurred. Median survival was 23 weeks for all patients measured from Day 1 of intrathecal chemotherapy.

Laboratory Parameters

In 13 of 17 (76%) evaluable patients, lumbar CSF was cleared of cancer cells for at least 1 month. In eight of 12 (66%) evaluable patients, malignant cells were eradicated from the ventricular CSF for > 1 month.

Hypoglycorrhachia was reversed in all nine patients in whom it was present in the initial lumbar CSF sample. Pleocytosis was present in lumbar CSF in 16 patients and in the ventricular CSF in eight patients and resolved in both sites in all patients.

Toxicity

Myelosuppression was the major toxic effect of this treatment. Nineteen (76%) patients developed leukopenia of < 2500 wbcs/mm^3 and in nine (36%) it was profound (< 1000 wbcs/mm^3). In 14 (56%) patients thrombocytopenia occurred (< 100,000 cells/mm^3); asymptomatic platelet counts < 10,000/mm^3 were seen in four. One death was due in part to granulocytopenia.

The myelosuppression experienced by these patients was attributable to concomitant systemic cytotoxic therapy and the irradiation of large volumes of bone marrow. Six patients received only intrathecal chemotherapy with or without cranial irradiation and none developed platelet counts < 100,000/mm^3. Two patients developed wbc counts < 2500/mm^3, and no patients had a wbc count < 1000/mm^3. All other patients received extensive radiation therapy (13 patients) and/or concomitant myelosuppressive therapy (nine patients).

CSF infections occurred in four patients (8). One infection occurred in a patient with a wbc count < 1000/mm^3. All infections were controlled with antibiotics and removal of the subcutaneous reservoir and intraventricular catheter.

Treatment-related neurotoxicity, characterized by memory loss and a broad-based shuffling gait, occurred in three patients. In two patients these findings partially resolved when intrathecal therapy was stopped, while in the third no improvement occurred.

Sixteen of the 25 patients have died. Progressive systemic cancer was the cause of death in six (38%) patients, while in another six (38%) death was due to a combination of progressive systemic and CNS malignancy. Toxicity related to treatment of neoplastic meningitis contributed to the death of two patients. In four (25%) patients death was due to uncontrolled CNS disease alone.

DISCUSSION

Gutin et al (9,10) have reported phase I–II trials indicating the safety and efficacy of intrathecal thiotepa in patients with refractory meningeal lymphoma and leukemia. Myelosuppression and neurotoxicity were noted in their trial. These data suggested the feasibility of combining thiotepa with methotrexate to investigate combination intrathecal chemotherapy.

Our study demonstrates that such combination therapy is feasible and effective. The chemotherapy itself caused only mild toxic effects. The major toxic effect in our series was myelosuppression, which was primarily due to the simultaneous administration of systemic chemotherapy and radiation therapy.

The role of intraventricular versus intralumbar administration of chemotherapy, the benefit of local radiation therapy, and the efficacy of thiotepa and methotrexate compared to "standard" intrathecal chemotherapy remain to be determined in neoplastic meningitis. Our data indicate that combination intrathecal chemotherapy utilizing an alkylating agent and an antimetabolite is safe and yields encouraging therapeutic results. Prospective trials comparing thiotepa and methotrexate to single-agent therapy will be required to determine the relative merits of these treatment plans.

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Brief Reports

Sequential Methotrexate and 5-FU in the Treatment of Colorectal Cancer[1]

B. Weinerman,* B. Schacter, H. Schipper, D. Bowman, and M. Levitt[2]

SUMMARY

Administration of methotrexate (MTX), 5-FU, and leucovorin rescue in 29 patients with metastatic colorectal cancer produced an objective response rate of 41.7%, with two complete remissions. Two patients developed fatal toxic effects and three others had severe toxic effects. This trial, however, supports the evidence for the synergistic effect of this drug combination.

[Cancer Treat Rep 66:1553–1555, 1982]

There are data suggesting that the combination of methotrexate (MTX) and 5-FU may be synergistic in the treatment of cancers (1–4). Theoretically, this is accomplished by shunting more 5-FU (5–8) into RNA production, where it may act more effectively. Furthermore, other data suggest that this synergistic effect may not be present for bone marrow cells, thereby increasing the therapeutic index of the agents (3). In view of these reports, we initiated a phase II trial to determine the response rate and the toxicity of MTX, 5-FU, and leucovorin rescue (LR) in the treatment of metastatic colorectal carcinoma with measurable disease.

METHODS

All patients, whether previously treated or not, were admitted to the trial if they had a creatinine clearance > 60 ml/minute, an Eastern Cooperative Oncology Group (ECOG) performance status < 4, and measurable disease.

Table 1 illustrates the chemotherapy protocol. All patients were well-hydrated and were alkalized with $NaHCO_3$ to keep the urine at a pH > 6.5 throughout the treatment.

No attempt was made to guide the total number of treatments given, although it was recommended that treatments be continued (minimum, two) until progressive disease intervened. Treatment was administered every 21–28 days if the wbc count was > 4000/mm^3, the platelet count was > 100,000/mm^3, and the creatinine clearance was > 60 ml/minute.

MTX levels were determined in several patients by the ^{125}I-MTX radioimmunoassay.

Response evaluation criteria included: *complete response (CR)*—complete tumor disappearance; *partial response (PR)*—a ≥ 50% reduction in tumor volume; *stable disease*—no change or < 50% reduction in tumor volume; and *progressive disease*.

RESULTS

When all 29 patients were included in the analysis, there were two patients (6.8%) with CR, eight (27.6%) with PR, and 11 (38%) with progressive disease. If only those patients who received two or more treatments were analyzed the CR rate was 8.3% and the PR rate was 33.3%; 33.3% of the patients had stable disease; and 25% had progressive disease. The median duration of remission was 4 months, and the median duration of stable disease was 5 months.

Five patients had one treatment, six had two, six had three, five had four, and seven had five or more treatments. There were a total of 100 treatments in 29 patients. Toxicity was noteworthy: 11.1% of the patients had a wbc count nadir < 1500/mm^3, and similarly, 11.1% had a platelet count nadir < 50,000/mm^3; 22.2% developed mucositis; 13.3% developed dermatitis; and 8.8% (four patients) had renal complications. Two patients died with severe dermatitis, mucositis, and leukopenia, with subsequent infection. Responding patients and those with stable disease survived significantly longer ($P < 0.001$) than nonresponding patients (fig 1). There was no significant survival difference between responders and those with stable disease (fig 1).

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[1] Received Oct 26, 1981; revised Feb 22, 1982; accepted Mar 9, 1982.

[2] Hematology-Oncology Service, Department of Medicine, University of Manitoba, Winnipeg, Canada.

**Reprint requests to:* Dr. B. Weinerman, Department of Medicine, St. Boniface General Hospital, 409 Tache Ave, Winnipeg, Manitoba R2H 2A6, Canada.

TABLE 1.—Protocol*

Time	Drug (dose)
Hour	
0	D5W† ½ normal saline (150 ml/hr) with $NaHCO_3$ (3 g) orally every 6 hrs for 30 hrs
3	MTX (20 mg/kg in 500 ml of D5W) infused continuously for 4 hrs
7	5-FU (600 mg/m²) iv bolus. D5W ½ normal saline (150 ml/hr) until the end of therapy at Hour 28
16	LR (10 mg/m² iv)
22	LR (10 mg/m² iv)
28	LR (10 mg/m² iv)
34	LR (10 mg/m² iv)

*Study criteria were normal BUN and creatinine, creatinine clearance > 60 ml/min, and ECOG performance status < 4. Treatments were repeated every 21–28 days.

†D5W = 5% dextrose in water.

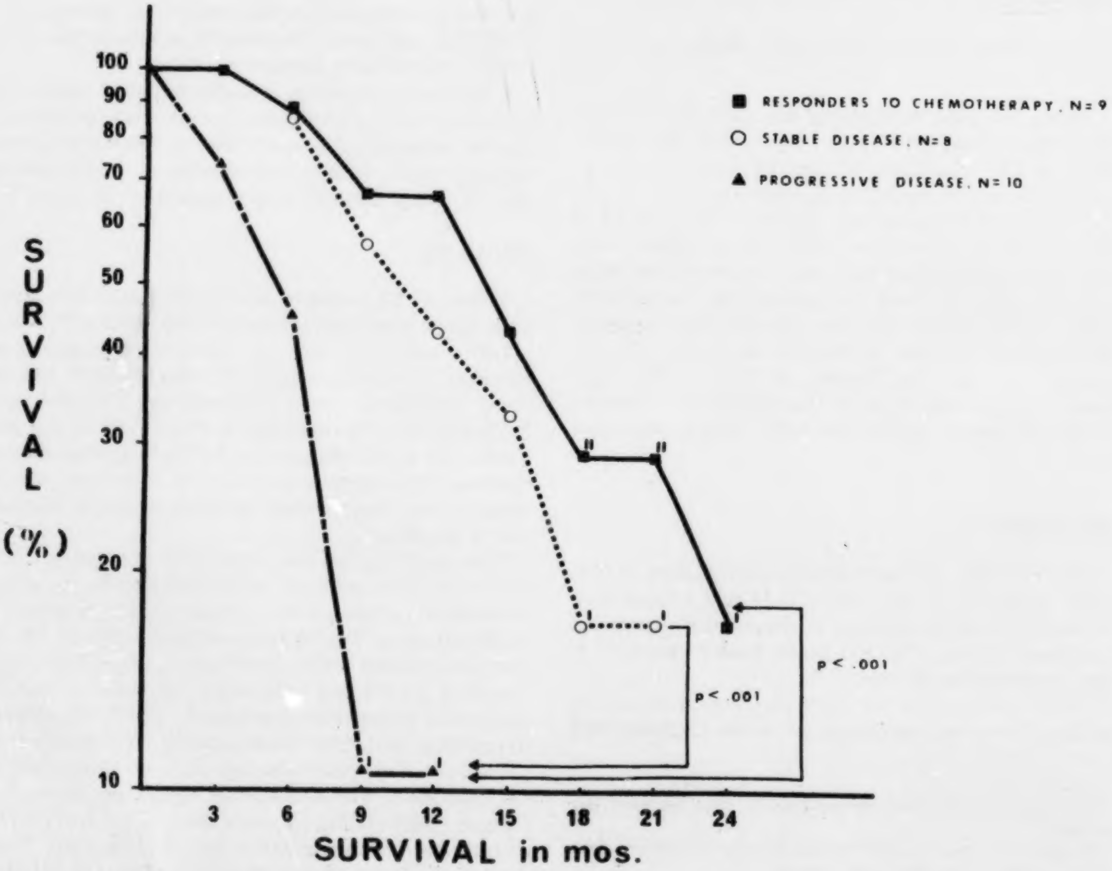

FIGURE 1.—Survival curve: metastatic colorectal cancer.

MTX levels were not measured in the five patients who developed severe toxic effects. The MTX levels that were determined were very low.

CONCLUSIONS

It appears that the combination of MTX, 5-FU, and LR as given in the series had a relatively high response rate (41.7%). This is supportive evidence for the synergistic effect of this combination. However, the toxicity was often severe and unpredictable. Two of the patients developed severe toxic effects during the first treatment, and the three others developed them in a subsequent treatment, despite adequate renal function and appropriate LR. MTX levels were not measured at the beginning of each treatment cycle, so that it is possible that MTX or 5-FU levels were still present from earlier treatments, although at least 3 weeks had elapsed between the treatment cycles. Remission durations were not long (4–5 months), but this trial was not rigidly designed to look at remission duration, so continued treatment in some of the patients may have lengthened remission and survival durations.

The utilization of MTX, 5-FU, and LR merits more exploration. Modifications in scheduling to increase response rates and lessen toxic effects must be tried. Longer treatment programs may maintain remissions longer. Care must be taken, however, in view of the toxicity of the program. Measurement of serum MTX levels appears to be mandatory in the attempt to elucidate toxicity mechanisms.

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CCNU, Vincristine, Methotrexate, and Procarbazine Treatment of Relapsed Small Cell Lung Carcinoma[1,2]

Brief Reports

Elizabeth A. Poplin,* Joseph Aisner, David A. Van Echo, Margaret Whitacre, and Peter H. Wiernik[3]

SUMMARY

Thirty-two patients with refractory relapsed small cell carcinoma of the lung (SCCL) were treated with a combination of CCNU (lomustine), vincristine, methotrexate, and procarbazine (COMP); 29 were evaluable for response. Nine patients (31%) had responses: five complete responses (CR) and four partial responses. Patients with CR had a median survival of 11 months (range, 5½–14½) from the start of COMP. Patients with less than CR had a median survival of approximately 3 months (range, < 1–7). The comparison of CR versus less than CR is significant (*P* = 0.003). Patients achieving CR usually had limited disease and four of the five who achieved CR survived > 6 months. The regimen was well-tolerated, but myelosuppression was seen in all patients. COMP appears to be a useful combination in patients with relapsed SCCL. Aggressive retreatment should be considered in relapsed patients with this disease since some may achieve a second CR, with the associated potential survival benefit.

[Cancer Treat Rep 66:1557–1559, 1982]

Despite recent improvements in the treatment of small cell lung carcinoma (SCCL) (1–3), most patients still relapse after successful primary treatment (4,5). With rare exceptions, patients who relapse or have refractory disease have rapidly progressive disease, with short survival irrespective of subsequent treatment (3,6,7). Hence, the current study was undertaken to evaluate the combination of the active agents CCNU (lomustine), vincristine, methotrexate, and procarbazine (8) in patients with recurrent or refractory disease previously treated with cyclophosphamide, doxorubicin, and etoposide.

MATERIALS AND METHODS

All patients who relapsed or had progressive disease while receiving primary treatment were eligible for study. All patients had previously received a combination of cyclophosphamide, doxorubicin, and etoposide (CAVP-16) (2) as initial therapy and had histologic proof of SCCL, recovery from the toxic effects of prior therapy, normal renal and hepatic functions (serum creatinine < 1.5 mg/dl and serum bilirubin < 1.5 mg/dl), and total wbc count > 3000/μl and platelet count > 100,000/μl.

All patients had a complete restaging evaluation including: physical examination, cbc and differential, serum chemistry, chest roentgenogram, scintiscans of bone and liver, computerized tomographic brain scan, and bilateral bone marrow aspirates and biopsies either at relapse or prior to protocol entry. Performance status was scored according to the Karnofsky scale, and extent of disease was scored according to the usual criteria. All patients evaluable for response had at least one measurable indicator lesion at the start of the protocol.

Chemotherapy on a 42-day cycle consisted of CCNU (75 mg/m^2) orally on Day 1; vincristine (1.4 mg/m^2; maximum dose, 2 mg) iv and methotrexate (40 mg/m^2) iv on Days 1 and 21; and procarbazine (75 mg/m^2/day) orally on Days 1–5 and 21–25 (COMP). If neutropenia or thrombocytopenia occurred at the time of re-treatment, therapy was usually delayed for 1 week, doses were reduced, or both. However, in instances of bone marrow involvement, no modifications were made. All patients were treated until relapse or disease progression, and there was followup of all patients until death. Radiation therapy was utilized during the course of COMP chemo-

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[1] Received Nov 9, 1981; revised Mar 15, 1982; accepted Mar 29, 1982.

[2] Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.

**Reprint requests to:* Elizabeth A. Poplin, MD, University of Maryland Cancer Center, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

therapy only for intracranial metastasis. Standard response criteria were employed (2). Durations of response and survival were measured from the start of COMP therapy.

RESULTS

Three of 32 patients entered in the study were considered inevaluable since they did not fulfill protocol entry requirements. Characteristics of the 29 evaluable patients are shown in table 1. When staged for primary therapy, 15 of the 29 patients had had limited disease and 14 had had extensive disease. At the time of COMP (relapse) therapy, eight patients had only local relapse or progression (limited disease) and 21 had extensive disease relapse.

Response

The overall complete and partial response rate (CR + PR) was 31%, with five patients (17%) achieving CR and four (14%) achieving PR. Eighteen patients did not respond to this regimen, and two had disease stabilization for approximately 2 months. Four of the five patients achieving CR had limited disease. None had received prior chest irradiation and three of the four patients with limited disease had previously achieved CR. Among the patients with extensive disease, one had CR and three had PR. Attainment of CR correlated with limited extent of disease ($P = 0.003$) but not with age, performance status, or duration or magnitude of response to previous chemotherapy. The median duration of response for the five patients achieving CR was 7.5 months (range, 3–10) compared to the median response of 3 months for those patients who achieved PR. Response was usually observed by the end of the first course, and responding patients received two to seven courses of chemotherapy. Twelve of the 18 nonresponding patients received only one course of COMP before showing disease progression. For these 29 patients with relapsed disease, there was no apparent correlation between the durations of the response to therapy with CAVP-16 and to the COMP treatment.

TABLE 1.—Characteristics of patients receiving COMP

Characteristic	No. of patients
Evaluable for—	
Response	29
Toxic effects	28
Median age in yrs (range): 60 (40–69)	
Sex	
Males	20
Females	9
Limited disease	8
Performance status	
80	4
70–80	11
70	14
Prior CAVP-16	29
Prior CNS radiotherapy	6
Prior non-CNS radiotherapy	10
Median duration in mos of response to prior therapy (range): 7.7 (2–20)	

Survival

Overall survival from initiation of COMP until death ranged from < 1 to 15 months. Patients with limited disease had a median survival of 6.5 months compared to 3 months for patients with extensive disease ($P = 0.003$). Patients who achieved CR had a median survival of 11 months (range, 5.8–15), while those who achieved PR had a median survival of 4 months (range, 3–6). Those with less than PR had a median survival of 2.5 months (range, < 1–7). Patients with CR survived longer than those with less than CR ($P = 0.003$). Four of the five patients achieving CR had survival > 6 months. Pretreatment performance status also correlated directly with survival ($P = 0.001$).

Toxicity

In the 28 patients evaluable for toxic effects, myelosuppression was most prominent. Dose reduction of myelosuppressive agents, usually to 50% of the scheduled dose, was required in nine of 17 patients receiving more than one course of chemotherapy. The median wbc count nadir of each patient during therapy was 2400/μl, and six patients had nadirs ≤ 1000/μl. The median platelet count nadir was 61,000/μl, and six patients had nadirs ≤ 20,000/μl. Patients with platelet counts < 20,000/μl received prophylactic platelet transfusions. Only one of 14 patients with platelet counts < 50,000/μl had a serious bleeding episode.

Other side effects included alopecia and nausea and vomiting. Mucositis was noted in one patient; one developed bilateral foot drop; and two, who had received radiation to the midchest and upper chest, experienced unilateral vocal cord paralysis. No hepatic or renal toxicity was noted. There were no drug-related deaths.

DISCUSSION

COMP was studied in 29 patients who had previously received chemotherapy, 13 of whom had also received radiotherapy. Contrary to the usual low response rate in patients with relapsed or progressive SCCL, COMP produced an overall response rate of 31%, with 17% CR, 14% PR, and survival > 6 months for five patients. The presence of limited relapse figured largely in these favorable results. Among the eight patients with limited relapse, four achieved CR. The overall median duration of survival for patients with limited relapse was 6.5 months, more than twice that seen in patients with extensive disease. The median survival for patients with CR was 11 months compared to the 3-month median survival in patients not achieving CR. This study suggests that COMP represents one therapeutic option for relapsed SCCL and that it is non-cross-resistant to the primary combination of cyclophosphamide, doxorubicin, and etoposide. At the time of initiation of this study, procarbazine was considered to be active in SCCL (8). However, since more recent data (9) have indicated that this drug may not be active, its inclusion in such future salvage regimens may be subject to reconsideration.

The results of this study suggest that for patients who relapse with limited disease and good performance status, survival may be significantly augmented with the use of the active second-line treatment. This may be of importance in patients for whom new drug investigation is not feasible. Furthermore, the identification of active second-line treatments may produce more active first-line treatments or regimens useful for alternating chemotherapy.

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Neurologic and Cardiac Toxicity Following Iv Application of Methotrexate[1]

Brief Reports

Adrian B. Gasser,[2,*] Marcel Tièche,[3] and Kurt W. Brunner[2]

SUMMARY

The case of a 61-year-old female patient with metastatic breast cancer is reported. She developed neurologic symptoms and supraventricular extrasystoles when she received iv methotrexate. These symptoms were reproducible and were not found with other drugs. The cause for this event is not clear.

[Cancer Treat Rep 66:1561–1562, 1982]

CASE REPORT

We have recently observed a patient who developed neurologic symptoms and cardiac arrhythmia following iv methotrexate (MTX) administration in conventional doses during treatment of metastatic breast carcinoma.

This 61-year-old female had had allergic bronchial asthma since 1968. She was also allergic to penicillin. In 1978 she underwent a mastectomy for an infiltrating ductal carcinoma of the left breast. The clinical stage was indicated as $T_2N_0M_0$. Postoperative radiotherapy was given.

The patient was hospitalized on March 23, 1981, reporting backache. The radiologic examination revealed metastases in the thoracic and lumbar spine, the pelvis, and the right femur. No other metastases were found. The physical examination was normal except for skeletal pain on percussion. The hematologic values were normal except for an unexplained eosinophilia found on hospital admission and persisting until May 23, when it disappeared spontaneously. It ranged from 12% to 16%, with a calculated absolute eosinophil count of 180–918/μl. Serum creatinine was normal (1.1 mg/dl), as was serum calcium (10 mg/dl). The alkaline phosphatase was elevated at 561 units/liter (normal up to 170). SGOT was also slightly increased at 42 units/liter as was SGPT at 73 units/liter. The ECG showed a sinus rhythm. The P-Q interval was shortened (0.11 secs) due to a delta wave in leads II, avF, V2, and V3. A left axis deviation was present. The cardiac silhouette was normal in a routine chest X-ray. Computed tomography of the brain showed no abnormalities. Cerebrospinal fluid was not examined.

Antidepressant therapy with imipramine (25 mg three times a day) and levopromazine (25 mg twice a day) was started on hospital admission and was continued during the whole period for reactive depression. One month later, on April 25, 1981, antitumor treatment was instituted orally with tamoxifen (20 mg/day), prednisone (50 mg/day), and cyclophosphamide (150 mg/day). In addition, weekly iv injections of 5-FU (1000 mg) and MTX (60 mg) were planned.

During the first injection of MTX the patient developed palpitations, dizziness, and nausea after 20 mg had been administered. Treatment was stopped immediately. An ECG was not done. A second attempt at MTX administration on the following day had to be interrupted again since the patient developed similar symptoms and an incomplete motor aphasia. The latter subsided over the following 3 days. On May 4, a third attempt at MTX administration was undertaken under cardiac monitor control. After 30 mg had been injected, multiple supraventricular extrasystoles were observed and the MTX was stopped. The blood pressure remained normal at 150/80. The patient reported an oppressed feeling and lost consciousness for 5–10 seconds. During this time muscle spasms were observed in the neck. The acute symptoms subsided within 3 hours. A mild motor aphasia persisted for 2 days. The ECG remained unchanged without further extrasystoles. It should be noted that the patient had received no medication prior to the second and the third MTX injections. Further attempts at MTX treatment were not made. Similar neurologic or cardiac episodes

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[1] Received Mar 15, 1982; accepted Mar 24, 1982.

[2] Institute of Medical Oncology, University of Bern, Inselspital, Switzerland.

[3] Medical Department, Bürgerspital, Solothurn, Switzerland.

*_Reprint requests to:_ Dr. A. B. Gasser, Cancer Research Campaign, Department of Medical Oncology, Christie Hospital and Holt Radium Institute, Wilmslow Rd, Manchester M20 9BX, England.

have not recurred during an observation period of 4 months, with otherwise unchanged treatment.

DISCUSSION

The hematologic toxicity of MTX is well-known. The drug has also been associated with stomatitis, alopecia, and hypersensitivity skin reactions. Cases of renal, hepatic, and pulmonary damage have also been reported (1). The wide variety of neurologic side effects reported in the literature have, however, only been observed following either intrathecal administration or carotid artery infusion (2). In a recent review of cardiac toxicity associated with antitumor therapy, descriptions of possible MTX-induced cardiac symptoms are mentioned (3): one patient died of congestive heart failure following bronchopneumonia due to leukopenia (4). Rosen et al (5) found a threefold increase in doxorubicin-induced ECG changes in patients previously treated with high-dose MTX and leucovorin rescue. Recently, a report of unusual MTX toxicity occurring in close temporal relationship with systemic MTX administration has appeared (6). Among others, the investigators relate three cases of loss of consciousness and one acute myocardial infarction.

In our patient, the MTX was given iv in conventional doses. On three successive occasions the MTX administration was followed by dramatic neurologic symptoms and cardiac arrhythmia. The ECG reverted to normal immediately following the third MTX injection and has remained so since. We cannot exclude an interaction between MTX and imipramine.

It would be difficult to explain the neurologic symptoms on the basis of transient cerebral ischemia. The systemic blood pressure remained normal throughout the entire period of observation before and after the MTX applications. There were no evident signs of inflammatory or metastatic lesions within the central nervous system, which could have altered the blood-brain barrier. The hypothesis of an anaphylactic reaction can be made, but we have no supportive evidence for it.

Although we cannot explain these rare reactions, their causal relation to MTX administration is highly probable. It therefore seems important to realize that such neurologic and cardiac side effects may occasionally follow iv injections of MTX in conventional doses.

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Phase II Study of Sequential Methotrexate-5-FU Therapy in Advanced Measurable Colorectal Cancer[1,2]

Brief Reports

James E. Cantrell, Jr,[3] Rene Brunet,[4] Claude Lagarde,[4] Philip S. Schein,[3] and Frederick P. Smith[3,*]

SUMMARY

Eighteen patients with previously untreated advanced colorectal cancer were treated with a sequential methotrexate-5-FU regimen. One of 16 evaluable patients achieved a partial response. The results of this and other clinical and laboratory investigations reported to date suggest that further study of different doses and schedules of sequential methotrexate-5-FU is warranted.

[Cancer Treat Rep 66:1563–1565, 1982]

A schedule-dependent cytotoxic synergism between methotrexate and 5-FU has been demonstrated by Bertino et al (1). Using a Sarcoma 180 mouse tumor model, they demonstrated a synergistic antitumor effect for the combination when the methotrexate was administered 1 hour before the 5-FU, whereas simultaneous or reverse sequence administration produced a survival effect similar to either drug given alone. They postulated that the pretreatment with methotrexate enhanced the cytotoxic action of 5-FU by increasing the amount of dTMP synthetase irreversibly bound by a ternary complex consisting of FdUMP, methotrexate, and dTMP synthetase. Cadman et al (2) confirmed the synergism in L1210 leukemia cell culture. They proposed a different mechanism of synergism based on methotrexate inhibition of purine utilization of phosphoribosyl pyrophosphate. Consequently, phosphoribosyl pyrophosphate accumulates and is diverted to use in the formation of 5-FU ribonucleotide compounds, which accumulate intracellularly, as well as in the parent drug 5-FU and FdUMP (2,3).

To date, a limited number of clinical trials using various drug doses and schedules of sequential methotrexate-directed 5-FU appear to confirm the efficacy for this approach in the treatment of breast cancer (4,5), head and neck cancer (6), and colorectal cancer (5,7,8). We report the results of a phase II trial of a sequential methotrexate-5-FU combination in previously untreated patients with advanced measurable colorectal cancer; the response rate observed was less than expected if 5-FU had been used alone.

PATIENTS AND METHODS

Eighteen consecutive patients with histologically documented metastatic colorectal carcinoma were treated with a methotrexate-5-FU sequential regimen. The median age was 57 years (range, 35–72), with a median Eastern Cooperative Oncology Group performance status of 1 (range, 0–3). The predominant sites of disease were lung (six patients) and liver (nine); other areas involved were bone, nodes, peritoneum, vagina-pelvis, and skin. None of the patients had received any prior chemotherapy. Patients had previously undergone radiation therapy to the pelvis (seven patients), bone (one), liver (one), and mediastinum (one), but none had received treatment to the site of followable disease.

The treatments consisted of 250 mg/m^2 of methotrexate followed in 1 hour by 600 mg/m^2 of 5-FU. Each drug was administered as an iv bolus. Leucovorin at a dose of 10 mg/m^2 was administered iv or orally 24 hours after the infusion of 5-FU and then orally every 6 hours for 2 days. Treatments were repeated at least every 3 weeks and usually within 2 weeks and were continued until disease progression occurred.

Response was assessed by clinical examinations,

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[1] Received Nov 12, 1981; revised Feb 19, 1982; accepted Mar 1, 1982.

[2] Supported in part by the French-American Agreement for Cancer Research.

[3] Division of Medical Oncology, Department of Medicine, Vincent T. Lombardi Cancer Research Center, Georgetown University, Washington, DC.

[4] Fondation Bergonie, Bordeaux, France.

[*] *Reprint requests to:* Frederick P. Smith, MD, Division of Medical Oncology, Georgetown University, 3800 Reservoir Rd, NW, Washington, DC 20007.

serial X-rays, and nuclear scans when appropriate. Patients were classified as having a complete response, partial response, stable disease, or progressive disease. Complete response was defined as the disappearance of all measurable disease for ≥ 2 months. Partial response was defined as a 50% reduction in the products of two perpendicular tumor diameters for ≥ 2 months. Progressive disease in other sites or the occurrence of new lesions negated a partial response. Stable disease was defined as either a < 50% reduction or a < 25% increase in tumor size for ≥ 2 months. Progressive disease was defined as a ≥ 25% increase in the size of measurable tumor mass or the appearance of new tumors. Survival was calculated from the day of initiation of chemotherapy. Toxicity was noted with each course and hematologic parameters were checked prior to each treatment.

RESULTS

Sixteen of the 18 patients were evaluated for response. One each was excluded due to no followable disease or lack of followup. One partial response was observed, as evidenced by a 50% reduction in vaginal-pelvic masses. This response was first noted after four treatments (10 weeks) and has persisted for > 2 months. Disease stabilization occurred in six patients and persisted for 10–30 weeks. The remaining nine patients experienced progressive disease. Median survival was 5 months (range, 2+ to 10) for the responder and for stable patients and 4 months (range, 1–5) for patients with progressive disease. Toxicity was limited to one episode of leukopenia (2300 wbcs/mm^3) and one case of mild mucositis.

DISCUSSION

This disappointing response with sequential methotrexate-5-FU chemotherapy in patients with previously untreated colorectal cancer stands in contrast to the reported response rates for colorectal and other malignancies treated similarly (table 1). There are a number of possible explanations for this apparent discrepancy.

While the results of the present clinical trial appear to be different from those reported from preceding studies, there are also conflicting experimental data relating to methotrexate-5-FU interactions. Tattersall et al (10) treated L5178Y cells in vitro with the combination and noted a less than additive effect with a schedule in which methotrexate was given 6 hours prior to 5-FU. In contrast to the results reported with L1210 tumor cells, Benz and Cadman (11) reported no synergism for the combination against a human colorectal adenocarcinoma in vitro during the first 6 hours after methotrexate exposure. Thereafter, a gradual increase in cell growth inhibition occurred, which was maximal during the last 6 hours of a 24-hour methotrexate exposure. It is therefore quite possible that if therapeutic synergism is to be achieved, it may require a very specific drug-drug interaction that is dependent on factors such as dose, schedule, and tumor specificity (12).

The 1-hour dosing interval used in this study was based on the published results of Bertino et al (1). However, their system used Sarcoma 180 mouse cells in a logarithmic growth phase and consequently may underestimate the time needed for a relatively slow-growing carcinoma to experience a comparable biochemical effect. Drapkin et al (8) and Herrmann et al (7) (table 1) achieved response rates of 33% and 60%, respectively, when they treated advanced colorectal carcinoma with a 5-FU dose comparable to that employed in the present report but with a lengthened pretreatment phase; methotrexate was administered as a 4-hour continuous infusion followed by a 3- or 7-hour delay until the 5-FU treatment. This schedule resulted in a 7–11-hour increase in methotrexate exposure time, which may have contributed to the superior results.

The dose of 5-FU employed in our treatment program may have been inadequate to exploit the full potential of this drug. The Central Oncology Group

TABLE 1.—Summary of clinical trial results

Clinical trial (ref No.)	Tumor	No. of patients	Response rate (%)
Cantrell et al (present study)	Colorectal	16	6
Tisman and Wu (5)	Colorectal	11	28
Herrmann et al (7)	Colorectal	10	60
Drapkin et al (8)	Colorectal	19	33
Gewirtz and Cadman (4)	Breast	17	53
Tisman and Wu (5)	Breast	13	23
Pitman et al (6)	Head-neck	10	100

(9) has presented results that support a high-dose loading regimen for the treatment of colorectal carcinoma with 5-FU alone. The sequential methotrexate-5-FU regimens of Tisman and Wu (5) and Herrmann et al (7) (table 1) against advanced colorectal cancer both achieved response rates superior to ours using higher doses of iv bolus 5-FU: 1500 mg/m² every 3–4 weeks and 900 mg/m² every 2–3 weeks, respectively. These results suggest that the higher doses of 5-FU are more effective.

Based on the results of our study, we conclude that the dose and schedule of sequential methotrexate-5-FU employed is not active against previously untreated advanced colorectal cancer. Future studies of this combination should be directed toward exploring larger doses of 5-FU and lengthening the time interval between administration of the drugs. Finally, regardless of which regimen modifications are employed, it appears appropriate to explore this approach in more drug-responsive tumors such as breast cancer.

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Brief Reports

Phase II Trial of Doxorubicin Therapy for Advanced Islet Cell Carcinoma[1,2]

Charles G. Moertel,* Philip T. Lavin, and Richard G. Hahn[2]

SUMMARY

Twenty evaluable patients with advanced islet cell carcinoma were treated with doxorubicin at a dosage of 60 mg/m² every 3–4 weeks. Toxicity was clinically tolerable and characteristic of doxorubicin therapy. Although all patients had previously been treated with other chemotherapy regimens, four (20%) had objective therapeutic responses persisting for 2–30½ months. Doxorubicin has definite therapeutic activity in islet cell carcinoma and is currently being evaluated in combination chemotherapy regimens.

[Cancer Treat Rep 66:1567–1569, 1982]

Islet cell carcinoma of the pancreas has a number of unique features including a usually indolent clinical course and a variety of endocrine hyperfunction syndromes that compound the effects of the carcinoma per se. In addition, this neoplasm presents unique opportunities for therapeutic exploitation, since a number of agents have been found in experimental animals to be specifically toxic to the parent cell. Of these, both tubercidin and streptozocin have been found to be active against human islet cell carcinoma, and the latter drug has an established clinical role. Streptozocin produces objective responses in only about 30% of patients however, and in general, these responses are only partial and relatively transient. This agent also has side effects that are unpleasant for most patients and intolerable for some. A clear need exists for additional active agents that may either supersede streptozocin in effectiveness and tolerability or combine with it to produce enhanced effectiveness. With the exception of scattered reports of 5-FU, there have been very few recorded studies in islet cell carcinoma with the more commonly used cytotoxic drugs, even those known to have a broad spectrum of activity. In 1976, the Mayo Clinic reported objective responses in two patients with islet cell carcinoma treated with doxorubicin (1). The present study was designed to expand that experience with the larger case-contributing potential of the Eastern Cooperative Oncology Group (ECOG).

METHODS

Patient Selection

All patients selected for study were required to have histologically proven locally unresectable or metastatic islet cell carcinoma. It was also required that each patient have measurable malignant disease to serve as an objective indicator of response to therapy. This indicator could be either tumor mass or a laboratory assay specifically related to endocrine hyperfunction, eg, serum gastrin. Malignant hepatomegaly was accepted as an indicator if the liver had been shown histologically to be metastatically involved and if a distinctly palpable liver edge extended at least 5 cm below the xiphoid process or costal margin on quiet respiration. A radioactive liver scan was accepted if it displayed a clearly demarcated perfusion defect measuring at least 5 cm in greatest diameter. Patients were excluded if they had severe malnutrition or frequent vomiting, leukopenia (< 4000 cells/mm³) or thrombocytopenia (< 150,000 cells/mm³), or any evidence of active heart disease. Therapy was delayed for at least 1 month

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[1] Received Dec 28, 1981; accepted Feb 23, 1982.

[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Participating institutions include: Mayo Clinic, Rochester, MN (*C. G. Moertel* and *R. G. Hahn*, CA-13650); Sidney Farber Cancer Institute, Boston, MA (*P. T. Lavin*, CA-23318); Albert Einstein College of Medicine, Bronx, NY (CA-14958); Baystate Medical Center, Springfield, MA (CA-20182); Case Western Reserve University, Cleveland, OH (CA-14548); Hahnemann Medical College, Philadelphia, PA (CA-13611); Mount Sinai Medical Center, New York, NY (CA-17152); National Cancer Institute, Bethesda, MD; University of Ottawa, Ontario, Canada; Centre Hospitalier de Bobigny, Paris, France (CA-19273); Swiss Group, Bern, Switzerland; and Yale University School of Medicine, New Haven, CT (CA-15485).

*__Reprint requests to:__ Charles G. Moertel, MD, Comprehensive Cancer Center, Mayo Clinic, Rochester, MN 55905.

after any previous radiation therapy or treatment with cytotoxic drugs.

Treatment Procedures

Doxorubicin was given by rapid injection into the tubing of an established iv infusion. Treatment was repeated at 3 weeks, at 6 weeks, and every 4 weeks thereafter. The dose for each treatment was 60 mg/m² unless the patient had an elevation of serum bilirubin. In the latter case, the initial dose was 40 mg/m², with escalation, if this dose was well-tolerated, to 60 mg/m² for continued therapy. Treatment was continued until there was evidence of disease progression or until a total doxorubicin dose of 500 mg/m² had been administered.

Therapeutic results were assessed prior to each scheduled dose of doxorubicin. An objective response was defined as reduction of a tumor by $> 50\%$ in the product of the longest perpendicular diameters. If malignant hepatomegaly was used as an indicator, the sum of measurements below the xiphoid process at each costal margin and at each costal margin at the midclavicular line had to be reduced by at least 30%. If a laboratory assay of endocrine hyperfunction was used, the abnormality had to be reduced by at least 50%.

RESULTS

A total of 25 patients were entered in the study. Three patients were declared ineligible, two because they did not have measurable parameters of malignant disease and one because therapy was not administered according to protocol method and dosage. Two patients were completely unevaluable for either toxic effects or response. Therapeutic results could therefore be evaluated in 20 patients. Their characteristics are presented in table 1. All but two patients had had disease progression after prior therapy with either streptozocin alone or streptozocin plus 5-FU. The remaining two patients had had disease progression after previous treatment with 5-FU alone. Even though all patients had had prior treatment, none were totally disabled, and 55% had an ECOG performance status of 0 or 1; ie, they were capable of working either full or part time.

Toxicity

Toxic effects were characteristic of doxorubicin therapy, with nausea or vomiting on the day of administration and the universal occurrence of alopecia. Moderate leukopenia was experienced by 47% of the 15 patients for whom adequate wbc counts were obtained to determine a nadir. In these patients, wbc count nadirs ranged from 2000 to 3900/mm³. One patient developed nonfatal congestive heart failure after receiving a total doxorubicin dose of 400 mg/m².

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Sex	
Male	12
Female	8
Median age in yrs (range): 54 (17–78)	
Performance status*	
0	2
1	9
2	6
3	3
Prior chemotherapy	
Streptozocin	18
5-FU	10
Semustine	2
Chlorozotocin	1
Tumor function	
None	10
Zollinger-Ellison syndrome	4
Insulinoma	3
Hypercalcemia	2
ACTH	1
Glucagon	1

*ECOG score: 0 (fully active) to 4 (totally disabled).

Therapeutic Results

Four (20%) of the 20 evaluable patients had an objective response according to the criteria above. Durations of response from the start of therapy were 2, 2, 6, and 30+ months. One of the 2-month responses was in a patient who refused to continue doxorubicin after his first dose because of nausea and vomiting. The longest response was still continuing at 30½ months, with treatment having been discontinued after the patient reached a limiting total dose of 500 mg/m². All responses occurred in patients with nonfunctioning tumors.

The median survival of all patients was 6 months from the start of doxorubicin therapy (range, 1½–36+ months).

DISCUSSION

This study has demonstrated that doxorubicin has definite activity in the treatment of advanced islet cell carcinoma. Its substantive activity is emphasized by the 20% response rate obtained in this group of patients, all of whom had had prior chemotherapy.

A previous ECOG study showed that the combination of 5-FU and streptozocin produced a significant improvement in response rate as well as in completeness of response compared to streptozocin alone (2). The present study indicates that, in islet cell carcinoma, doxorubicin has activity that probably at least matches that of 5-FU. Pilot studies conducted at the Mayo Clinic have shown that a nearly full dose of doxorubicin can be combined with a full dose of streptozocin. The current ECOG study involves a randomized comparison of the doxorubicin-streptozocin combination, the 5-FU-streptozocin combination, and chlorozotocin used alone.

Although it is a rare neoplasm, the islet cell carcinoma is clearly vulnerable to a chemotherapeutic attack, and substantial increase in patient survival should be an obtainable goal.

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Brief Reports

In Vitro Evaluation of the Cardiotoxic Potential of AMSA[1]

Michael C. Lowe[2,*]

In contrast to many other drug therapies, cancer chemotherapy is seldom limited by therapeutic response but more often by systemic toxicity. Because the low therapeutic indices of most oncolytic agents derive from extensions of the intended effect of these drugs (ie, the killing of cells) (1), oncologists have by necessity learned to clinically manage most of the untoward responses. However, an atypical toxicity, such as the chronic cardiotoxicity of doxorubicin or daunorubicin, is much more difficult to assess and reverse, especially when it may not become apparent until several months after treatment (2).

Recently, there have been reports of a possible cardiotoxicity caused by AMSA, an acridine currently in clinical trials. There have not yet been any reported chronic cardiotoxic reactions with AMSA, but there have been cases where acute, life-threatening cardiac arrhythmias have occurred following administration of the drug (3). The electrophysiologic effects of AMSA appear to be similar to the early reports of cardiac problems following daunorubicin administration (nonspecific electrocardiographic changes with no histologic evidence of myocardial damage) (4,5). It was not until some years later that the full spectrum and incidence of cardiotoxic reactions to doxorubicin and daunorubicin were realized (ranging from acute electrophysiologic changes to a chronic cardiomyopathy characterized by myocytolysis, vacuolization, and, ultimately, congestive heart failure) (2,6).

With the slowly developing story of anthracycline cardiotoxicity in mind, it was decided to test the effects of AMSA on isolated rat heart myocytes, cells that have recently been shown to be sensitive to several anthracyclines (7).

MATERIALS AND METHODS

Cardiac myocytes were isolated from male Sprague-Dawley rats (250–300 g) as previously described (7) and were stored in Ca^{2+}-free buffer until used (within 24 hours). Summarily, the isolation procedure required a Langendorff perfusion of the heart with Ca^{2+}-free buffer containing hyaluronidase and collagenase, followed by several digestion and purification steps. AMSA (bulk) was obtained from the Drug Development Program of the National Cancer Institute and was dissolved in a 40% (vol/vol) solution of dimethyl sulfoxide (DMSO; Fisher Scientific Co) in glass-distilled water. Test concentrations of AMSA were prepared at four times the desired final concentration and were used within 1 hour. Microscopic determinations of the maximal durations of spontaneous contractions of the isolated myocytes ($1–3 \times 10^6$ cells/ml) were made in a calcium-containing buffer (Tyrode's) on hemacytometer slides at room temperature. Control observations were made with drug-free cells in Tyrode's buffer with or without 10% DMSO (the final concentration used with drug-treated cells). Up to six determinations were made at each concentration or condition tested except when there appeared to be no effect (> 70 minutes) or when the effect was so drastic it was impossible to find any beating cells (< 1 minute). Maximal durations of beating times are reported as mean values ± SD when more than two determinations were made.

RESULTS

AMSA was soluble in 40% DMSO and distilled water up to approximately 1×10^{-3} M, although yellow crystals of AMSA appeared in the solution within 30 minutes. At 1×10^{-4} M, AMSA appeared to stay in solution for > 6 hours; occasionally, while maximal beating times were being determined, crystals of AMSA would be observed on the microscope slides, in which case the experiment was terminated.

Control cells in Tyrode's buffer beat for > 70 minutes, confirming earlier observations; the addition of

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[1] Received Dec 3, 1980; revised Mar 3, 1982; accepted Mar 25, 1982.

[2] Toxicology Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* Michael C. Lowe, PhD, Toxicology Branch, National Cancer Institute, National Institutes of Health, Bldg 37, Rm 5A13, Bethesda, MD 20205.

DMSO (final concentration, 10% [vol/vol]) to the buffer did not affect control beating times. When cells were exposed to 1×10^{-3} M AMSA, it was not possible to find any contracting cells among approximately 4000 cells on each test slide. Similarly, AMSA at 8×10^{-4} M and 6×10^{-4} M concentrations prevented the cells from contracting. When less concentrated solutions of AMSA were tested, a dose-response relationship was observed, but over a very narrow concentration range (table 1). Whereas 1×10^{-4} M AMSA was indistinguishable from non-drug controls (beating times > 70 minutes), 4×10^{-4} M AMSA stopped all contractions within 5 minutes. Contracting cells simply ceased to beat, arresting in various states or configurations (some cells were elongated; others were obviously supercontracted).

DISCUSSION

AMSA has been found to be toxic to spontaneously contracting isolated rat heart myocytes. Compared to doxorubicin or daunorubicin in the same test system, AMSA is approximately one tenth as potent, having an ID50 (that concentration at which the last contracting cell would cease to beat at one half the control maximal beating time) of 1.7×10^{-4} M versus 1.5×10^{-5} M for doxorubicin or daunorubicin (which are indistinguishable in this system).

Although the mechanism(s) by which anthracyclines affect the isolated heart cells used in this study is unknown, the relative specificity of the effect has been intriguing. Other anticancer drugs (methotrexate, mitomycin, 5-FU, etc) and agents known to be extremely toxic to cells in tissue culture (ie, dactinomycin) were nontoxic when tested (7).

Cardiovascular pharmacology studies of AMSA in dogs (8) predicted the acute electrophysiologic effects of the drug in man, and hemodynamic responses were only seen at high doses. In an attempt to determine the chronic cardiomyopathic potential of AMSA, the drug was tested in rabbits with weekly injections over 12 or 26 weeks (9). At the dose (determined by an acute LD50 determination) studied, AMSA was found to be marginally cardiotoxic (two of six high-dose animals had mild histologic changes in the heart). It is possible that the dose of AMSA given in the study (one tenth of the LD50 weekly) was too low and was not sufficient to show a chronic doxorubicin-type of cardiotoxicity because of the overriding acute (electrophysiologic) toxicity of AMSA.

TABLE 1.—Dose-response effects of AMSA on maximal durations of beating times of isolated adult rat heart myocytes

Group (No. of determinations)	Maximal duration of beating, in mins (mean ± SD)
Controls	
Tyrode's buffer (2)	> 70
10% DMSO:90% Tyrode's buffer (2)	> 70
AMSA in 10% DMSO:90% Tyrode's buffer	
1×10^{-4} M (6)	71.6 ± 12.3
2×10^{-4} M (6)	32.0 ± 9.7
4×10^{-4} M (6)	4.5 ± 1.4
6×10^{-4} M (2)	< 1
8×10^{-4} M (2)	< 1
1×10^{-3} M (2)	< 1

Both dog and rabbit studies used the clinical formulation of AMSA, which contains *N,N*-dimethylacetamide (DMA) as a solvating agent. Von Hoff et al (3) have raised the possibility that DMA might contribute to the electrophysiologic effects observed with AMSA. Although that question was not addressed in this study, DMA was obviously not responsible for the observed effects of AMSA on heart cells, since the clinical formulation was not used.

The similarity of AMSA to the anthracyclines in the isolated myocyte system should perhaps inject a note of caution into the clinical use of AMSA. Until it is known by what mechanisms anthracyclines act acutely (electrophysiologically) or chronically (cardiomyopathically), it must be considered that AMSA might be acting in a similar way. The fact that early reports of anthracycline cardiotoxicity centered about acute electrophysiologic effects (that were discerned long before the later developing congestive heart failure syndrome) should alert oncologists to watch for cardiac performance changes in patients receiving AMSA.

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Effects of Interferon on C1300 Mouse Neuroblastoma[1,2]

Brief Reports

Mutsuhiko Tozawa,* Takuro Kidowaki, Terufusa Tanaka, Tadashi Sawada, Tomoichi Kusunoki, Tohru Oku, and Tsunataro Kishida[3]

SUMMARY

The effects of murine interferon (MuIFN) with and without cyclophosphamide (CY) were evaluated in C1300 neuroblastoma-bearing mice. Mean survival times were prolonged by administration of 25,000 IU of MuIFN three times per week by ip injection. The combination of MuIFN and CY was more effective in the treatment of mouse neuroblastoma than either agent alone. Our results suggested that MuIFN is synergistic in combination with moderate amounts of CY in mouse neuroblastoma cells and may be given for the treatment of neuroblastoma.

[Cancer Treat Rep 66:1575–1577, 1982]

Most cases of neuroblastoma have an unfavorable prognosis in spite of intensive treatment regimens. New regimens are necessary to improve the prognosis for patients with this tumor.

In 1974, Strander et al (1) suggested that human leukocyte interferon had some beneficial effects on patients with osteosarcoma. Thereafter, interferon was experimentally administered to patients with various malignant diseases (2,3). In animal experiments, Gresser et al (4) showed that murine interferon (MuIFN) was effective on spontaneous lymphomas in AKR mice and that the combination of MuIFN and cyclophosphamide (CY) was more effective in tumor therapy than either MuIFN or CY alone.

In this paper, we report a study of the antitumor effects of MuIFN and the combination of MuIFN and CY on C1300 neuroblastoma-bearing A/J mice.

MATERIALS AND METHODS

Mice and tumor.—Inbred A/J female mice, 6–12 weeks old, were used in this study. A syngeneic C1300 neuroblastoma tumor was maintained by serial transfers in vivo. For this study, A/J mice were inoculated with 1×10^6 C1300 neuroblastoma cells sc in the interscapular area.

Murine interferon.—MuIFN was prepared at our laboratory for this study. It was induced on a monolayer of L_{929} cells infected with Newcastle disease virus (Miyadera strain). The specific activity of the L cell was 1.4×10^6 IU/mg of protein (5).

Experiment.—Four groups were prepared for this study (fig 1). In group 1, the control group, each mouse was treated with a 0.25-ml saline solution three times each week. Group 2 was given 20 mg/kg of CY. Group 3 was treated with MuIFN at a dose of 25,000 IU/mouse three times each week. Group 4 was given a combination of MuIFN and CY at the same doses as groups 2 and 3. Saline, MuIFN, and CY were administered by ip injection. These treatments were started on Day 8 after inoculation of tumor cells, when a palpable mass could be detected, and were continued until death of the animal. This experiment terminated on Day 75 after inoculation of tumor cells. The survival times of the two living mice in group 4 were estimated to be > 75 days. The significant differences in mean survival times for each group were tested by Student's t test.

RESULTS

The effects of MuIFN and/or CY administration on tumor-bearing mice were evaluated by survival time and incidence of tumor regression in each group compared with those of the control group. The re-

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[1] Received Feb 5, 1981; revised Mar 15, 1982; accepted Mar 30, 1982.

[2] Supported by a grant-in-aid for cancer research from the Ministry of Health and Welfare, Japan.

[3] Departments of Pediatrics (*M. Tozawa, T. Kidowaki, T. Tanaka, T. Sawada,* and *T. Kusunoki*) and Microbiology (*T. Oku* and *T. Kishida*), Kyoto Prefectural University of Medicine, Japan.

**Reprint requests to:* Mutsuhiko Tozawa, MD, Department of Pediatrics, Kyoto Prefectural University of Medicine, Kawaramachi, Hirokoji, Kamikyoku, Kyoto, Japan 602.

Group	Treatments	No. of Mice	Survival days (mean SD)*	Survivors on day 70
1	Saline,0.25mlx3/W	8	24.1± 1.9	0/8
2	CY, 20mg/kgx1+Saline	7	30.3± 2.9	0/7
3	L-cell IFN,25,000IUx3/W	7	33.0± 3.2	0/7
4	L-cell IFN+CY	8	>50.7± 5.4	2/8

* Significance of difference of mean survival days; (1)vs(3):$p<0.05$, (1)vs(4):$p<0.001$, (2)vs(4):$p<0.01$ and (3)vs(4):$p<0.02$.

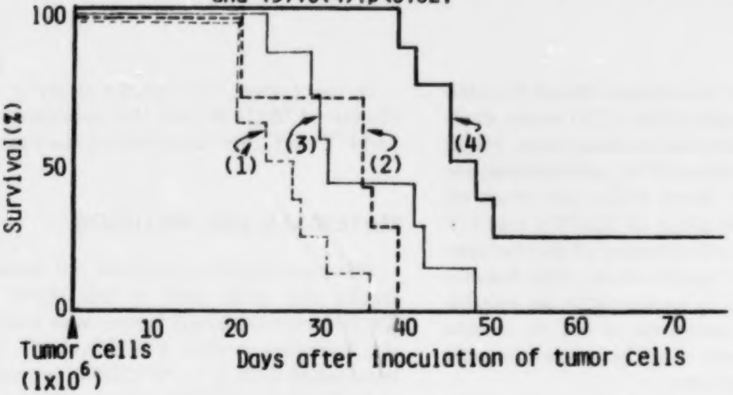

FIGURE 1.—Effects of MuIFN and CY on survival times of C1300 neuroblastoma-bearing mice.

sults are shown in figure 1. MuIFN was effective in prolongation of survival of the tumor-bearing mice ($P < 0.05$). Although 20 mg/kg of CY was considered to be effective in prolonging survival, there was not enough statistical evidence ($P > 0.05$). Group 4, receiving combination therapy with MuIFN and CY, showed a more prolonged survival than either of the groups receiving MuIFN or CY alone. Total regression of the tumor mass was noted in two of the eight mice of group 4; those two animals survived for > 75 days after inoculation of tumor cells.

DISCUSSION

This study was the first trial of MuIFN administration to C1300 neuroblastoma-bearing mice. In this study, administration of MuIFN and/or CY was started on Day 8 after inoculation of 1×10^6 tumor cells, when a palpable mass could be detected. A preliminary study was carried out using MuIFN of different origins and different doses of MuIFN or CY. Two MuIFNs were prepared for this study: L-cell MuIFN and brain MuIFN (extracted from the brains of ICR mice inoculated with Japanese encephalitis virus [JaGAr 01]). Survival times (mean ± SE) of the five tumor-bearing mice treated with 25,000 IU of brain MuIFN and L-cell MuIFN (28.2 ± 4.5 and 29.4 ± 3.0 days, respectively) were longer than the mean survival time of five mice treated with 2500 IU of brain MuIFN (23.3 ± 4.4 days). However, the difference between survival times after doses of 25,000 and 2500 IU of MuIFN was not significant. There was also no statistical difference in the mean survival period with MuIFNs from different origins.

In the preliminary studies of chemotherapy for neuroblastoma, a high dose of CY (230 mg/kg) was reported to be the most effective dose in prolonging the survival of C1300 neuroblastoma-bearing A/J mice (6). Since many mice died from side effects of the high dose of CY, it was reduced to 20 and 40 mg/kg for this study. There was no significant difference in mean survival between the group receiving 20 mg/kg of CY and the control group; however, seven tumor-bearing mice injected with 40 mg/kg of CY had prolonged mean survival times ($P < 0.01$). As a result, to eliminate the harmful side effects of CY and to evaluate the effects of combination therapy with MuIFN and CY, the CY dose was reduced to 20 mg/kg.

Tumor masses of two group 4 mice totally regressed after combination therapy, and both mice were alive at the termination of this study.

These results showed that combination therapy with MuIFN and CY may be a favorable new regimen, and the mechanism of regression of C1300 neuroblastoma is now being studied.

There have been two studies on the effects of combination therapy with MuIFN and a cytotoxic drug for leukemia and lymphoma. Chirigos and Pearson (7) reported the striking efficacy of MuIFN and carmustine (BCNU) for treatment of murine leukemia. Gresser et al (4) reported the efficacy of MuIFN and CY for treatment of spontaneous AKR lymphoma. Our combination MuIFN-CY therapy also showed success in the treatment of C1300 neuroblastoma. Doses of MuIFN and CY were lower than those in the previous report (4); however, our combination showed more inhibitory effects on tumor growth than either MuIFN or CY alone. We concluded that the prolonged survival of tumor-bearing mice resulted from synergistic effects of MuIFN and CY on tumor growth.

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Parenteral Formulation of Hexamethylmelamine Potentially Suitable for Use in Man[1,2]

Brief Reports

Matthew M. Ames* and John S. Kovach[3]

SUMMARY

We have developed a preparation of hexamethylmelamine (HMM), dissolved in a fat emulsion Intralipid 20%, that is potentially satisfactory for parenteral administration to man. Solid HMM can be dissolved in Intralipid 20% up to 5 mg/ml, with extensive agitation. Solubilization of HMM is facilitated first by dissolving the drug in ethanol or in dimethylacetamide and then by adding the solution to Intralipid 20%. The disposition of HMM in rabbits after iv administration of HMM dissolved in Intralipid 20% was similar to the disposition of the drug following iv administration of HMM dissolved in 0.1 N HCl.

[Cancer Treat Rep 66:1579–1581, 1982]

Hexamethylmelamine (HMM) is an antitumor agent with activity against several human malignancies (1). Because of the lack of a suitable parenteral preparation, HMM has been administered orally to man. Oral administration of HMM to rabbits (2) and to man (3) results in low and variable concentrations of drug in plasma. We have demonstrated that the low concentration of HMM in plasma after oral administration to rabbits results from extensive metabolism by the liver (first pass effect) rather than from poor absorption from the gut (2). There are no quantitative data concerning the absorption of HMM in man.

Several parenteral preparations of HMM have been studied. These include the free base dissolved in 0.1 N HCl and the hydrochloride and gentisate salts dissolved in water. The acid vehicle produced moderate local irritation and venous thrombosis in large animals (4). The gentisate salt of HMM produced severe local irritation in animals (5) and was unstable at physiologic pH (6). The administration of HMM hydrochloride iv to beagle dogs was reported recently to be well-tolerated (7). However, in a limited clinical trial conducted in 1962, the hydrochloride salt caused severe local irritation and phlebitis in man (B. Morrision, III, National Cancer Institute [NCI]: memo to operating committee).

The NCI recently developed pentamethylmelamine (PMM), a water-soluble analog of HMM, for clinical testing by parenteral administration (5). HMM and PMM have been reported to have comparable activity in vivo against transplantable tumors in rodents (5). PMM administered iv to man in phase I trials did not produce significant cytotoxicity at doses causing intolerable gastrointestinal and central nervous system toxicity (8–12). To our knowledge, no phase II trials of PMM are planned. Because we believe that the antitumor activity of parenterally administered HMM should be evaluated in man, we have been attempting to develop a formulation of HMM suitable for iv administration. We report that HMM in Intralipid 20% appears suitable for testing as a parenteral formulation of HMM in man.

MATERIALS AND METHODS

Intralipid 20%, a fat emulsion consisting of 20% soy bean oil, 1.2% egg yolk phospholipids, and 2.25% glycerine in water, was purchased from Cutter Laboratories, Berkeley, CA. HMM was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI, Bethesda, MD. Filters (0.45 μ) were purchased from Millipore Filter Company, Milford, MA.

The hydrochloride salt of HMM was prepared by dissolving HMM in ether and bubbling gaseous HCl through the ether solution followed by filtration and recrystallization of the precipitated salt. HMM and its hydrochloride salt were dissolved in aqueous solution or in Intralipid 20% by shaking for 2 hours

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[1] Received Oct 5, 1981; revised Feb 26, 1982; accepted Mar 8 1982.

[2] Supported in part by American Cancer Society grant CH 143. Dr. Ames is the recipient of National Cancer Institute Research Cancer Development Award CA-00755 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic, Rochester, MN.

**Reprint requests to:* Matthew M. Ames, PhD, Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic, Rochester, MN 55905.

in screw-top centrifuge tubes at room temperature. HMM was also dissolved in ethanol at 5, 10, and 20 mg/ml and in dimethylacetamide (DMA) at 20, 40, and 50 mg/ml. The HMM-ethanol or HMM-DMA solutions were added to a stirred solution of Intralipid 20%. The concentration of HMM was measured by a nitrogen-phosphorus gas chromatographic assay following extraction of alkalinized aliquots of the aqueous and lipid solutions with toluene as previously reported (13).

New Zealand white rabbits (2.5–3.1 kg) were injected iv in an ear vein with HMM dissolved in a minimal volume of 0.1 N HCl, HMM dissolved in Intralipid 20%, or HMM dissolved in ethanol or DMA and then added to Intralipid 20%. Samples of blood were collected in heparin tubes from the uninjected ear.

RESULTS

The maximum amount of HMM which could be dissolved in water was 0.20 mg/ml. The hydrochloride salt of HMM dissolved readily in water or 0.9% sodium chloride up to 20 mg/ml. The pH of solutions of HMM hydrochloride in water or 0.9% sodium chloride ranged from 3 to 2 at concentrations from 1 to 20 mg/ml, respectively. Measurement of HMM in toluene extracts of Intralipid 20% after addition of known amounts of solid HMM to the lipid preparation showed almost complete dissolution (allowing for extraction efficiency) at a theoretic concentration of 5 mg/ml (89.5% ± 6.6%). HMM was not completely dissolved in Intralipid 20% at a theoretic concentration of 10 mg/ml (58.4% ± 4.9%).

To facilitate complete and reproducible dissolution of HMM in Intralipid 20%, we dissolved HMM in ethanol (14) or in DMA. HMM dissolved in ethanol with warming to 37°C up to 20 mg/ml. At 10 mg/ml in ethanol, HMM remained in solution at room temperature for at least 2 hours. At 20 mg/ml in ethanol, HMM remained in solution at room temperature for at least 10 minutes. HMM dissolved in DMA at room temperature up to 50 mg/ml. Addition of HMM in ethanol at 20 mg/ml to Intralipid 20% achieved a maximal concentration of 2 mg/ml of HMM (10% ethanol) without disturbance of the homogeneity of the emulsion. Addition of HMM in DMA at 50 mg/ml to Intralipid 20% achieved a maximal concentration of 2 mg/ml of HMM (4% DMA) without disturbance of the emulsion. Analysis of toluene extracts of HMM in the ethanol and Intralipid 20% solution and in the DMA and Intralipid 20% solution showed quantitative recovery of drug after filtration through a 0.45-μ filter. Recovery of HMM from the DMA preparation was 95% ± 1.0% and from the ethanol preparation, 93.0% ± 0.6%. Samples stored at room temperature for 24 hours were unchanged in their content of HMM.

We studied the disposition of HMM in rabbits after iv administration of the lipid preparations. Peak plasma concentrations and rates of elimination of HMM from plasma were similar for the three preparations, and virtually identical to the characteristics of the drug when given dissolved in dilute HCl (3). Irritation of the ear of rabbits receiving the drug was always noted after the injection of acidic formulations of HMM, but not after injection of any of the three lipid preparations.

DISCUSSION

A preparation of HMM dissolved in Intralipid 20% appears suitable for iv administration to man. Fat emulsions and related systems have been used previously for parenteral administration of lipid-soluble antitumor agents including lomustine (CCNU), carmustine (BCNU), semustine (methyl-CCNU), bleomycin, and 5-FU (14–16).

Rapid and complete dissolution of HMM in the lipid was facilitated by first dissolving the drug in ethanol or in DMA. Either of these formulations appears suitable for iv administration to man. Administration of 0.5 g of HMM would necessitate administering 25 ml of alcohol (250 ml of 10% ethanol-lipid mixture containing 2 mg/ml of HMM) or 10 ml of DMA (250 ml of 4% DMA-lipid mixture containing 2 mg/ml of HMM), amounts of these two solvents which have been administered safely to man (17,18). Concentrations > 2 mg/ml of HMM in Intralipid 20% can be achieved by dissolving solid HMM in the lipid directly. Because the opacity of the lipid prevents visual assessment of the completeness of dissolution, it will probably be necessary to first dissolve HMM in one of the two solvents and then add the completely dissolved drug to Intralipid 20%.

We believe HMM administered iv should be evaluated for antitumor activity in man. HMM, but not its water-soluble metabolite PMM (8–12), has been demonstrated to be active against several human cancers. We have demonstrated that NADPH-dependent covalent binding of HMM in vitro is five times greater than NADPH-dependent covalent binding of PMM to microsomal macromolecules (19,20), a difference which may be related to antitumor activity of HMM compared to PMM. We have also documented in rabbits that the large first pass effect limits markedly the plasma concentration of HMM achievable after oral administration (2). For these reasons we believe that parenteral HMM may produce pharmacologic effects at much lower total doses than are customarily given orally. This parenteral formulation of HMM may also be of use for parenteral administration of HMM via routes other than iv, as has been tested in the treatment of advanced ovarian carcinoma.

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Clinical Trials Summaries

Phase II Trial of Prednimustine in Children With Recurrent Cancer: A Children's Cancer Study Group Report[1,2]

Paul S. Gaynon,* Edward S. Baum, William Krivit, and Denman Hammond[2]

Prednimustine is an ester of chlorambucil and prednisolone. Early trials in adults showed activity in breast cancer, chronic lymphocytic leukemia, acute myeloblastic leukemia (AML), non-Hodgkin's lymphoma (NHL), ovarian carcinoma, and prostatic carcinoma. The Children's Cancer Study Group designed a phase II trial, CCG-083, largely restricted to acute leukemia and NHL, where adult data suggest activity.

Of 141 patients entered, 99 were evaluable. The patients, all previously treated, received one of four regimens: *regimen D*—40 mg/m²/day for 28 days minimum; *regimen E*—40 mg/m²/day for 14 days with escalation to 60 mg/m²/day for 14 days minimum; *regimen I/200*—200 mg/m²/day for 5 days every 14 days; and *regimen I/300*—300 mg/m²/day for 5 days every 14 days. Seventy patients had acute lymphoblastic leukemia (ALL), 21 had AML, four had NHL, two had neuroblastoma, one had chronic myelogenous leukemia (CML) in blast cell crisis, and one had hepatocellular carcinoma (table 1).

In ALL, 12 objective responses were seen. Ten of these were minimal responses; eight patients had transient clearance of peripheral blast cells and two had markedly decreased organomegaly, with no improvement in marrow status. Two patients had PR, with reductions in the marrow from > 25% blast cells to between 5% and 25% blast cells. Neither response lasted 4 weeks. There were no CR seen in the marrow (< 5% blast cells). Both 60 mg/m²/day and 200 mg/m² on the intermittent schedule were adequate to suppress counts independently of clearing blast cells from the bone marrow.

The one patient with CML achieved an M2 marrow that lasted 4 months with regimen E. The one patient with hepatoma obtained a > 50% reduction in his primary tumor, as shown by computerized tomographic scan, that lasted 6 months with regimen I/200. The PR was accompanied by an increased sense of well-being.

Toxic effects included nausea and vomiting, hematemesis, abdominal pain, elevated transaminases with or without elevated bilirubin, and rashes. Of note, ten patients had seizures: eight grand mal and two myoclonic. In four patients the seizures were associated with intracranial hemorrhage; in six pa-

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[1] Received Nov 25, 1981; revised Mar 23, 1982; accepted Apr 1, 1982.

[2] This study was conducted by the Children's Cancer Study Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a Vancouver Foundation grant. The following institutions and investigators participated in the study: University of Southern California and Comprehensive Cancer Center, Los Angeles (Group Operations Office) (*D. Hammond*, *R. Honour*, *H. Sather*, *R. Sposto*, and *J. Weiner;* CA-13539); University of Michigan Medical Center, Ann Arbor (*R. Heyn;* CA-02971); Children's Hospital National Medical Center and George Washington University, Washington, DC (*S. Leikin;* CA-03888); Memorial Sloan-Kettering Cancer Center and Cornell University, New York, NY (*D. R. Miller;* CA-14557); Children's Hospital of Los Angeles and University of Southern California (*G. Higgins;* CA-02649); Babies Hospital and Columbia University, New York, NY (*J. Wolff;* CA-03526); Children's Hospital of Pittsburgh and University of Pittsburgh, PA (*V. Albo;* CA-07439); Children's Hospital of Columbus and Ohio State University (*W. Newton;* CA-03750); Children's Orthopedic Hospital and Medical Center and University of Washington, Seattle (*R. Chard;* CA-10382); University of Wisconsin Hospital, Madison (*N. Shahidi;* CA-05436); University of Minnesota Health Sciences Center, Minneapolis (*M. Nesbit;* CA-07306); Children's Memorial Hospital and Northwestern University, Chicago, IL (*G. Honig;* CA-07431); University of Utah Medical Center, Salt Lake City (*E. Lahey;* CA-10198); Strong Memorial Hospital and University of Rochester, NY (*M. Klemperer;* CA-11174); University of British Columbia, Vancouver, Canada (*M. Teasdale;* Vancouver Foundation grant); Children's Hospital of Philadelphia and University of Pennsylvania (*A. Evans;* CA-11796); James Whitcomb Riley Hospital for Children and Indiana University, Indianapolis (*R. Baehner;* CA-13809); Harbor General Hospital and University of California-Los Angeles, Torrance (*J. Finklestein;* CA-14560); University of California Medical Center, San Francisco (*A. Ablin;* CA-17829); Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, OH (*P. Coccia;* CA-20320); Children's Hospital Medical Center, University of Cincinnati, OH (*B. Lampkin;* CA-26126); University of Oregon Health Sciences Center, Portland (*R. Neerhout;* CA-26044); University of Texas Health Science Center, San Antonio (*P. Zeltzer*); University of Iowa Hospitals and Clinics, Iowa City (*C. T. Kisker;* CA-29314); Children's Hospital of Denver, CO (*D. Tubergen;* CA-28851); and Mayo Clinic, Rochester, MN (*G. Gilchrist;* CA-28882).

**Reprint requests to:* Paul S. Gaynon, MD, The Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614.

tients no etiology was determined. Two patients died with interstitial pneumonia: one had received only 2 days of therapy; the other had received 120 mg/m^2/day for > 1 month.

Both seizures (1,2) and interstitial pneumonia (3,4) have been reported with chlorambucil administration. The abdominal pain and hematemesis may be attributed to prednisolone side effects.

The uniqueness of prednimustine is confounded by the observation of Kirdani et al (5) that the drug is broken down quantitatively in the gut and no intact ester may be detected in the blood at any time. The lesser myelosuppression is due to lesser, if unpredictable, absorption. The unpredictability of absorption may be the cause of the seizures observed primarily in children receiving regimens I/200 and I/300.

TABLE 1.—Patient characteristics: response and toxicity

Characteristic			No. of patients	
Entered			141	
Evaluable for response			99	
Age in yrs				
Median: 9				
Range: 10 mos–23 yrs				
Sex				
Male			91	
Female			50	
Responses/trials for ALL	*MR*	*PR*	*CR*	*Trials**
Regimen D	3	1	0	19
Regimen E	5	1	0	27
Regimen I/200	1	0	0	13
Regimen I/300	1	0	0	11
Toxic effect				
Nausea and vomiting			17	
Abdominal pain			9	
Elevated transaminases			8	
Diarrhea			5	
Hematemesis			4	
Hypertension			3	
Rash			2	
Interstitial pneumonia			2	
Seizures			10	
Regimens D and E			1	
Regimens I/200 and I/300			9	

*MR = minimal responses; PR = partial responses; and CR = complete responses.

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Clinical
Trials
Summaries

Phase II Trial of Combination Methyl-GAG and Vinblastine in the Treatment of Metastatic Renal Adenocarcinoma[1,2]

Robert F. Todd, III, Marc B. Garnick,* and George P. Canellos[3,4]

Metastatic renal adenocarcinoma remains largely resistant to effective systemic therapy including chemotherapy. In a phase I–II trial, we recently reported that methyl-GAG administered weekly produced an objective response rate of 16% (one complete and three partial responses) in a series of 25 patients with this disease (1). A similar experience was reported by Knight et al (2). Myelosuppression was an infrequent drug-related side effect in these studies. Lack of this toxic reaction prompted a followup phase II trial consisting of methyl-GAG plus another marginally active agent, vinblastine (3,4), whose major dose-limiting side effect is myelosuppression. In this report, we describe our clinical experience using combination methyl-GAG and vinblastine in the treatment of metastatic renal adenocarcinoma.

Table 1 shows the pretreatment characteristics of 15 evaluable patients with measurable metastatic renal adenocarcinoma who were entered in the protocol. Two of the four patients who had received prior chemotherapy had been started on single-agent methyl-GAG for 3 and 4 weeks immediately before entrance in the combination protocol (pending approval of the combination study). No patients had received prior vinblastine. The treatment plan consisted of vinblastine administered by bolus iv infusion at a dose of 5 mg/m^2 on Day 1 of a 21-day treatment cycle; methyl-GAG was given by bolus iv infusion (over 45–60 mins) at a dose of 500 mg/m^2 on Days 2 and 8. At the start of this study, three patients were treated with vinblastine at a dose of 5 mg/m^2 on Days 1 and 2, but this resulted in unacceptable myelosuppression in two individuals (wbc count $< 500/\text{mm}^3$), prompting the subsequent administration of vinblastine on Day 1 only. The appearance of significant toxic reactions (table 1) resulted in discontinuation of both drugs until toxicity abated, followed by dose reduction of 10% (if wbc count was > 1000 but $< 2000/\text{mm}^3$ and platelet count

TABLE 1.—Patient characteristics, response, and toxic reactions in phase II trial of methyl-GAG and vinblastine in the treatment of advanced renal adenocarcinoma

	No. of patients
Patients evaluable (male/female)	15 (13/2)
Median age in yrs (range): 59 (16–76)	
Median performance status (range): 1 (0–2.5)	
Prior treatment	
Nephrectomy	13
Chemotherapy (doxorubicin, vincristine, dactinomycin, methyl-GAG)	4
Hormone therapy (Megace, Provera)	4
Radiation therapy (localized)	10
Sites of measurable disease	
Lung	13
Soft tissue mass	6
Bone	2
Liver	1
Skin	1
Median No. of courses of methyl-GAG + vinblastine (range): 3 (1–11)	
Partial or complete responses	0
Toxic reactions	
Nausea/vomiting	2
Weight loss (> 10% baseline)	5
Neuralgia/myalgia	6
Mucositis	2
Balanitis	1
Myelosuppression (total)	10
Leukopenia (< 3000 wbcs/mm^3)	7
Thrombocytopenia (< 100,000 platelets/mm^3)	1
Anemia (transfusions required)	6

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[1] Received Nov 30, 1981; revised Mar 22, 1982; accepted Apr 5, 1982.

[2] Supported in part by contract N01-CM-67037 (Division of Cancer Treatment) and Public Health Service grant CA-09172 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Division of Medical Oncology, Sidney Farber Cancer Institute; Department of Medicine, Harvard Medical School, Boston, MA.

[4] We thank Dr. Emil Frei, III, for his support throughout the study and the following physicians for referring patients to the study: G. Bowers, G. Hasler, M. Soloway, G. Strauss, and S. Weitzman.

**Reprint requests to:* Dr. Marc B. Garnick, Department of Medicine, Sidney Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

was > 50,000 but < 100,000/mm^3) or 50% (if wbc count was < 1000/mm^3, platelet count was < 50,000/mm^3, and severe nausea and vomiting as well as severe neuromuscular symptoms existed). All patients receiving a minimum of one complete 21-day cycle of therapy were evaluable for response and toxicity. Pertinent studies documenting extent of disease were repeated at 3–6-week intervals.

Using the accepted criteria for response (1), there were no documented complete or partial responses in this group of 15 evaluable patients (table 1). Toxic effects attributed to therapy are indicated in table 1; myelosuppression was the major toxic reaction, affecting 67% of the patients treated.

Based on this experience with these 15 evaluable patients, combination chemotherapy with methyl-GAG and vinblastine as administered according to this dose and schedule regimen does not improve on the minimal efficacy of these when given as single agents (4,5) in the treatment of metastatic renal adenocarcinoma.

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Phase II Evaluation of Maytansine in Refractory Non-Hodgkin's Lymphoma: A Southwest Oncology Group Study[1,2]

Clinical
Trials
Summaries

Voravit Ratanatharathorn,[3] Nazli Gad-el-Mawla,[4] Henry E. Wilson,[5] John D. Bonnet,[6] Saul E. Rivkin,[7] and Robert Mass[8,*]

Maytansine is a naturally occurring ansa macrolide originally isolated by Kupchan et al (1) from the East African shrub *Maytenus ovatus* and later from the wood and bark of *Maytenus buchananii*. The mechanism of action involves inhibition of mitotic spindle formation (2). In the experimental tumor screen, it was found to have significant activity against P388 leukemia, B16 melanoma, and Lewis lung carcinoma. A phase I study conducted at Wayne State University using a weekly schedule demonstrated tolerable toxicity, and maytansine was adopted for group-wide clinical trials in refractory non-Hodgkin's lymphoma (3).

Nineteen patients were entered in the study. Maytansine was administered by iv bolus at a dose level of 0.75 mg/m^2/week in 16 patients. For the three poor-risk patients, defined as those with abnormal liver function (values of transaminase, alkaline phosphatase, or bilirubin more than three times normal) or previous exposure to vinca alkaloid with residual disabling neuropathy, the dose was decreased to 0.5 mg/m^2/week. Subsequent doses were escalated by an increment of 0.25 mg/m^2 if no toxicity was encountered after 4 weeks of treatment. All patients with non-Hodgkin's lymphoma had been previously treated with a combination of cyclophosphamide, doxorubicin, vincristine, and prednisone; of these, 13 had also been treated with various combinations that contained several other drugs including methotrexate, cytarabine, bleomycin, a nitrosourea, and DTIC.

Criteria for response were defined as follows: *complete response*—the disappearance of all clinical evidence of active tumor, and *partial response*—a $\geq 50\%$ reduction in the sum of the products of the perpendicular diameters of all measured lesions. Response must have been maintained for at least 4 weeks to have been considered significant.

Two patients were excluded from evaluation due to early death from rapidly progressive disease. An average of five weekly injections were administered (range, two to 12 injections). Of the 17 evaluable patients, three received a starting dose of 0.5 mg/m^2, and 14 received 0.75 mg/m^2. Subsequent doses were escalated from 0.75 to 1.0 mg/m^2 in four patients and from 0.5 to 0.75 mg/m^2 in one patient. Only one patient had dose de-escalation because of thrombocytopenia after four weekly treatments of 0.75 mg/m^2. The median wbc and platelet count nadirs were 5.4×10^3/mm^3 (range, $1.4–21.2 \times 10^3$) and 160×10^3/mm^3 (range, $19–323 \times 10^3$), respectively. Two patients developed severe myelosuppression; both received 0.75 mg/m^2 for four and eight doses. Other toxic effects included weakness and paresthesia in three patients (who received four, five, and 12 weekly doses); the latter patient also developed severe diarrhea. One additional patient had diarrhea after receiving only two doses. All three patients who developed neuropathy had received four or more doses; two of these were started at 0.5 mg/m^2 because of pre-existing hepatic dysfunction.

However, neuropathy was not seen in four patients who received subsequent dose(s) of 1.0 mg/m^2 after four weekly injections of 0.75 mg/m^2, although none had received more than two escalated doses. Cumulative neurologic toxicity was not apparent but seemed to be related to pretreatment liver function abnormality. Partial remission was seen in one patient, lasting for only 4 weeks.

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[1] Received Dec 7, 1981; revised Mar 17, 1982; accepted Mar 29, 1982.

[2] Supported in part by Public Health Service grants CA-14028, 03-054-N, CA-04920, CA-20319, CA-21116, CA-04915, CA-13238, CA-22416, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Wayne State University, School of Medicine, Detroit, MI.

[4] Cairo National Cancer Institute, Egypt.

[5] Ohio State University Hospitals, Columbus.

[6] Scott and White Clinic, Temple, TX.

[7] Seattle Tumor Institute of the Swedish Hospital Medical Center, WA.

[8] University of Oregon Health Sciences Center, Portland.

**Reprint requests to:* Southwest Oncology Group (SWOG-7821), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

We therefore conclude that maytansine has no significant antitumor activity in heavily pretreated patients with non-Hodgkin's lymphoma.

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Phase II Trial of AMSA in Previously Treated Patients With Stage III and IV Ovarian Cancer[1,2]

R. J. Schneider, T. M. Woodcock, J. Howerd, and M. Ochoa, Jr[3,*]

AMSA is an acridine derivative whose cytotoxic effects are believed to be mediated by intercalation with, and external binding to, DNA (1). The drug was originally selected for clinical trials because it is an active antitumor agent for a diverse group of murine tumors including B16 melanoma, L1210 and P388 leukemias, and CD breast and Lewis lung carcinomas (2). Phase I clinical trials conducted at several centers demonstrated activity of AMSA in a variety of human neoplasms, including acute leukemia, breast cancer, and ovarian cancer (3–5). We have conducted a phase II investigation of AMSA in patients with International Federation of Gynecology and Obstetrics stage III and IV ovarian cancer who had previously been treated with chemotherapy.

PATIENTS AND METHODS

Twenty patients with histologically confirmed metastatic ovarian cancer were treated with AMSA. Entry in the study began in February 1979 and was concluded in March 1980. In all cases, pathologic material was reviewed by the Department of Pathology, Memorial Sloan-Kettering Cancer Center. Complete initial medical histories and physical examinations were recorded on standard evaluation forms. Automated blood and platelet counts were obtained weekly; performance statuses (Karnofsky scale) and screening biochemical profiles (including liver functions tests) were evaluated at least once every 3 weeks; and chest X-rays and radionuclide scans were obtained every 3–6 weeks, where indicated, to evaluate response.

Criteria for patient selection included acceptable hematologic status (leukocyte and platelet counts ≥ 4500 and 175,000/mm^3, respectively), the presence of bidimensionally measurable lesions, performance status $\geq 50\%$, and no evidence of significant renal or hepatic dysfunction (serum creatinine < 2.0 mg/100 ml; serum bilirubin < 1.5 mg/100 ml).

The median age for the 20 patients treated was 62 years (range, 47–75) and the median performance status was 80% (range, 50%–100%). Fifteen patients were previously treated with chemotherapy alone, and five had previously received chemotherapy and radiation therapy. All prior treatment had been discontinued for at least 3 weeks prior to the initiation of treatment with AMSA.

Measurable lesions consisted of abdominal and pelvic masses (14 patients), subcutaneous metastases (five), metastatic nodal disease (four), and hepatic (four) and pulmonary (three) metastases.

AMSA was administered as an iv infusion over a 30-minute period once every 3 weeks. The initial dose was 120 mg/m^2 for patients with good marrow reserve, while patients who had received extensive pelvic irradiation or multiple courses of chemotherapy with substantial prior bone marrow toxicity were begun at a dose of 90 mg/m^2. The dose was increased from 90 to 120 mg/m^2 if the leukocyte and platelet count nadirs were ≥ 3000 and $\geq 100{,}000$/mm^3, respectively. The dose was reduced from 120 to 90 mg/m^2 if the leukocyte and platelet count nadirs were < 3000 and $< 100{,}000$/mm^3, respectively.

An adequate trial was defined as two doses of AMSA with myelosuppression or one dose with myelosuppression and rapid disease progression. Response criteria were as follows: *partial remission*—a $\geq 50\%$ reduction in the sum of the products of the largest diameters of all measurable lesions for ≥ 1 month; *minor remission*—a $\geq 25\%$ but $\leq 50\%$ decrease in measurable lesions for ≥ 1 month or a $\geq 50\%$ decrease for < 1 month; *stable disease*—a $< 25\%$ increase or decrease in tumor size; and *disease progression*—a $\geq 25\%$ increase in the sum of the products of the largest diameters of all measurable lesions.

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[1] Received Dec 8, 1981; accepted Feb 2, 1982.

[2] Supported in part by contract N01-CM-57043 (Division of Cancer Treatment) and Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Developmental Chemotherapy (*R. J. Schneider, T. M. Woodcock,* and *J. Howerd*) and Solid Tumor (*M. Ochoa, Jr*) Services, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

[*] *Reprint requests to:* M. Ochoa, Jr, MD, Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

Table 1.—Hematologic toxicity of AMSA

			Leukocyte count nadir (cells × 10^3/mm^3)		Platelet count nadir (cells × 10^3/mm^3)	
Dose (mg/m^2)	No. of patients	No. of courses	Median (range)	Day	Median (range)	Day
90	13	20*	2.7 (0.9–7.3)	7	156 (70–346)	8
120	13	20†	1.9 (0.3–3.2)	14	125 (20–218)	14

*Additional 2 courses not evaluable due to lack of interim counts.
†Additional 3 courses not evaluable due to lack of interim counts.

RESULTS

All patients had an adequate trial. Thirteen patients received 90 mg/m^2, and 13 received 120 mg/m^2. Toxic effects included nausea and vomiting, which were mild and occurred in one half of the patients treated, and myelosuppression, which occurred at both dose levels (table 1). There was no evidence of renal or hepatic injury secondary to treatment with AMSA as reflected in measurements of serum BUN, creatinine, bilirubin, alkaline phosphatase, and SGOT. None of the patients responded to treatment with AMSA. As of July 1980, the median survival time from initiation of protocol to followup or death was 7 months (range, 1–17+).

CONCLUSIONS

This trial of AMSA in 20 patients with advanced ovarian cancer has produced significant myelosuppression without beneficial therapeutic effect. The results suggest that AMSA is an inactive agent in ovarian cancer.

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Clinical
Trials
Summaries

Phase II Evaluation of Gallium Nitrate in Breast Cancer: A Southwest Oncology Group Study[1,2]

Carol J. Fabian,[3] Laurence H. Baker,[4] Clarence B. Vaughn,[5] and Harry E. Hynes[3,*]

Gallium nitrate is a II_A metal salt which has been shown to concentrate in breast cancer tissue and hormonally stimulated normal breast tissue (1,2). We embarked on a phase II study of gallium nitrate in previously treated patients with breast cancer following phase I studies which indicated that 700 mg/m^2 every 2 weeks was a safe dose [(3); M. K. Samson, unpublished data].

MATERIALS, METHODS, AND RESULTS

All 26 heavily pretreated patients with metastatic breast cancer who were entered in the study were eligible. Eligibility requirements included measurable disease, life expectancy $\geq$ 6 weeks, BUN $\leq$ 20 mg/100 ml, creatinine $\leq$ 1.2 mg/100 ml, creatinine clearance $>$ 60 ml/minute, two functioning nonobstructed kidneys, bilirubin $<$ 2 mg/100 ml, no evidence of hyperuricemia or hypercalcemia, and no concomitant chemotherapy or radiotherapy. Patients were given 700 mg/m^2 of gallium nitrate in 250 ml of normal saline over 30 minutes following prehydration with 1500 ml of 5% dextrose in one-half normal saline every 2 weeks. An adequate trial was considered to consist of two courses of therapy.

Three of the 26 eligible patients received less than two courses of therapy (early death) and were considered to be partially evaluable. Among the 23 patients considered to be fully evaluable, there were no responses. The median number of courses of gallium nitrate received was three (range, two to nine courses), and the median performance status was 2 (symptomatic but ambulatory). Of the patients who might be expected to respond, ie, two with positive gallium scan, two who were estrogen-receptor positive, and one who was premenopausal, all developed progressive disease.

Toxic reactions among all 26 partially and fully evaluable patients were minimal, with five patients developing mild nausea and six developing minimal renal dysfunction (BUN $>$ 20–$<$ 40 mg/100 ml and/or creatinine $>$ 1.2–$<$ 2 mg/100 ml). One patient developed severe renal dysfunction preterminally due to tumor. Median survival for all patients was 26 weeks.

DISCUSSION

Despite reports of uptake of gallium in patients with breast cancer (1), particularly hormonally stimulated tissue (2), gallium nitrate did not show any significant activity in this heavily pretreated patient population.

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[1] Received Dec 14, 1981; revised Feb 16, 1982; accepted Mar 17, 1982.

[2] Supported in part by Public Health Service grants CA-12644, CA-14028, CA-04915, CA-13238, CA-22411, CA-03096, CA-16957, CA-04920, CA-03389, CA-20319, CA-16385, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] University of Kansas Medical Center, Kansas City.

[4] Wayne State University School of Medicine, Detroit, MI.

[5] Providence Hospital, RI.

[*] *Reprint requests to:* Southwest Oncology Group (SWOG-7917), Operations Office, 4450 Medical Dr San Antonio, TX 78229.

Case Report: Acute Leukemia After Semustine (Methyl-CCNU) Therapy[1]

Second malignancies have been the subject of many investigations involving patients previously treated for malignant lymphoma and myeloma. Radiation therapy and alkylating agents are among the major therapeutic regimens implicated. There have been several recent reports of acute leukemia following the administration of the nitrosoureas. The following is a case report of a patient who developed acute nonlymphocytic leukemia after treatment with semustine (methyl-CCNU) and 5-FU for Dukes' C adenocarcinoma of the colon. This patient had a prolonged complete remission after intensive antileukemia chemotherapy.

The patient was a 68-year-old female who had a right hemicolectomy on April 21, 1977, for a Dukes' C adenocarcinoma of the cecum. She then received adjuvant chemotherapy with a total dose of 1170 mg of semustine and 29.25 g of 5-FU between June 13, 1977, and May 22, 1978. A followup examination in April 1980 showed a hemoglobin of 13.9 g/dl, a wbc count of 4800/mm^3, and a platelet count of 150,000/mm^3. Differential count showed 61% segmented neutrophils, 1% bands, 29% lymphocytes, 8% monocytes, and 1% eosinophils. She was admitted to the hospital on July 21, 1980, with a 1-day history of gum bleeding. Cbc on admission showed a hemoglobin of 7.7 g/dl, a wbc count of 3000/mm^3, and a platelet count of 28,500/mm^3. Differential count showed 9% segmented neutrophils, 6% bands, 58% lymphocytes, 2% monocytes, 3% eosinophils, 10% blast cells, and 9% metamyelocytes. Her only medication had been hydrochlorothiazide for hypertension. A bone marrow examination the day after admission demonstrated 90% cellularity, with 50% of nucleated cells being blast cells; a diagnosis of acute myelomonocytic leukemia (FAB M-4) was made. Chromosome studies demonstrated that chromosomes 3, 5, 6, 17, and 21 were missing, and there was an extra C chromosome similar to 22. She was treated with doxorubicin, vincristine, prednisone, and a 7-day infusion of cytarabine (Southwest Oncology Group protocol) on July 28, 1980. Repeat marrow examinations on Days 7 and 9 of treatment showed a persistent cellularity of 90%, and the patient received cytarabine for 5 more days.

The patient received another course of induction chemotherapy, including 7 days of cytarabine, on September 13, 1980, after a marrow examination had demonstrated 10% myelomonoblasts. Subsequent marrow examinations demonstrated a complete remission of her leukemia; a cbc on January 30, 1981, showed a wbc count of 8700/mm^3 with a normal differential count, hemoglobin of 12.9 g/dl, and a platelet count of 85,000/mm^3. After 12 months in complete remission and while receiving late intensification therapy with POMP (6-mercaptopurine, vincristine, methotrexate, and prednisone), the patient relapsed. She subsequently died during the reinduction chemotherapy.

DISCUSSION

In a recent bulletin to physicians, the National Cancer Institute reported on two cases of acute leukemia, two cases of smoldering leukemia, and one case of aplastic anemia arising approximately 1 year after completion of adjuvant semustine therapy in the Gastrointestinal Study Group protocol No. 6175 for colorectal carcinoma. There were a total of 280 patients treated in this protocol, with a median followup of 34–35 months. In the control arm of the protocol, no cases of leukemia were reported.

Other reports of acute leukemia after nitrosourea therapy include the development of acute myelocytic leukemia in a 69-year-old male who was treated over a 28-month period with only 67 g of streptozocin for metastatic islet cell carcinoma (1). The patient was observed to have progressive leukopenia which terminated in acute leukemia 13 months after the streptozocin therapy was stopped.

Cohen et al (2) reported two cases of acute nonlymphocytic leukemia associated with nitrosourea chemotherapy. The first case was a 56-year-old female who was treated for angioblastic meningioma initially with a total of 720 mg/m^2 of carmustine (BCNU), subsequently with 700 mg/m^2 of lomustine (CCNU), and finally with 10,000 mg/m^2 of teniposide (VM-26) between January 1970 and October 1973. She developed acute myelomonocytic leukemia in December 1974, which was preceded by several months of progressive leukopenia. The second case was a 60-year-old female who received a total of 2700 mg/m^2 of semustine for a left frontal oligodendroglioma. No other chemotherapy was given.

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[1] Supported in part by Public Health Service grants CA-17984 and CA-15655 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

She developed progressive leukopenia during semustine therapy. This therapy was stopped, and 10 months later acute myelomonocytic leukemia was diagnosed.

In summary, therapy with nitrosoureas, especially if prolonged, has been associated with the development of acute leukemia. The leukemia may develop about 1 year after therapy has been discontinued and very frequently is preceded by a myelosuppressive phase. Survival after leukemia transformation has been notably very short, except in the case reported here. Of interest is the observation of a much higher risk of chemotherapy-related leukemia in patients > 40 years old compared to those < 40 (C. Coltman, personal communication).

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William J. Uphouse
Noboru Oishi*

University of Hawaii at Manoa
1236 Lauhala St, Rm 301
Honolulu, HI 96813

February 18, 1982

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*To whom reprint requests should be sent.

Experience of the Eastern Cooperative Oncology Group With Doxorubicin as a Single Agent in Patients With Previously Untreated Breast Cancer[1]

Various doxorubicin-containing combinations have been investigated over the past decade as alternatives to CMF (cyclophosphamide, methotrexate, and 5-FU) and, more recently, CMFP (CMF plus prednisone) combination chemotherapy for advanced breast cancer. Most of these trials involved the combination of doxorubicin and vincristine with or without other agents. However, a cohort of 19 patients initially treated with doxorubicin alone have not previously been reported, although brief reference to them has been made (1).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Performance status	
0–1	12
2–3	7
No. of sites involved	
1	2
2	9
≥ 3	8
Dominant site	
Soft tissue	1
Osseous	6
Visceral	12
Tumor-free interval (yrs)	
< 2	15
2–5	3
> 5	1

In 1973, the Eastern Cooperative Oncology Group (ECOG) initiated a trial of doxorubicin at a dose of 60 mg/m^2 given iv every 3 weeks to patients with advanced breast cancer not previously treated with chemotherapy. Standard ECOG criteria for treatment modification based on hematologic toxicity and for assessment of response were used. Ages of patients ranged from 37 to 75 years (median, 54). Patient characteristics shown in table 1 demonstrate a predominance of visceral involvement, short tumor-free intervals, multiple site involvement, and some limitation in performance status.

Eight patients (42%) responded: two with complete responses and six with partial (> 50% reduction in tumor diameter) responses. Four patients (21%) received only one course of therapy and were considered to have disease progression: one patient died 25 days after the first dose from unknown cause prior to reevaluation, one adrenalectomized patient died 10 days after the first dose from adrenal insufficiency precipitated by doxorubicin-induced vomiting, and two patients refused to continue treatment after one course (one due to doxorubicin-induced tachyarrhythmias).

The durations of response were 43, 63, 71, 139, 216, and 307 days for partial responses and 192 and 280 days for complete responses. Thus, the median duration of response was 5.5 months.

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[1] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Participating institutions include: Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-10948); Sydney Farber Cancer Institute, Boston, MA (CA-23318); Albert Einstein College of Medicine, Bronx, NY (CA-14958); University of Alberta, Edmonton, Canada; American Oncologic Hospital, Philadelphia, PA (CA-18281); Brown University, Providence, RI (CA-15947); Hahnemann Medical College, Philadelphia, PA (CA-13611); Jackson Memorial Hospital, Miami, FL; University of Florida-Jacksonville (CA-19818); Jefferson Medical College, Philadelphia, PA (CA-14215); University of Manitoba, Winnipeg, Canada; Mount Sinai Medical Center, New York, NY (CA-17152); Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Rochester Cancer Center, NY (CA-11083); Tufts University, Walpole, MA (CA-07190); and Medical College of Virginia, Richmond (CA-10572).

Hoogstraten and Fabian (2) have recently reviewed the reported activity of single agents in the treatment of breast cancer. The composite response rate to doxorubicin was 40% (49 of 126 patients) for patients not previously treated with cytotoxic chemotherapy. The reported duration of response has been generally shorter than that of more recently studied drug combinations. The response rate of 42% for a duration of 5.5 months in the 19 patients reported here is consistent with that observation. It is noteworthy that in the next generation of studies, the combination of doxorubicin and vincristine produced a slightly higher response rate of 56% for a duration of 7.7 months in a comparable group of 166 patients not previously treated with chemotherapy (3).

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Samuel G. Taylor, IV*
Richard D. Gelber

Eastern Cooperative Oncology Group

March 8, 1982

**Reprint requests to:* Samuel G. Taylor, IV, MD, Section of Medical Oncology, Rush-Presbyterian-St. Luke's Medical Center, 1725 W Harrison, Suite 830, Chicago, IL 60612.

Pharmacology and Toxicity of Ip Streptozocin in Ovarian Cancer: A Case Report[1]

Streptozocin, composed of the cytotoxic moiety 1-methyl-1-nitrosourea attached to the carbon-2 position of glucose, produces diabetes mellitus in mice secondary to the destruction of pancreatic beta cells. Concentration of streptozocin in the beta cells of the mouse pancreas has been demonstrated, suggesting that the glucose carrier of streptozocin facilitates uptake into the islet cells (1). Streptozocin has demonstrated clinical antitumor activity against islet cell carcinomas (2). However, the mechanism of transport of streptozocin has not been directly investigated.

In this preliminary report we describe the toxicity and pharmacokinetics of ip administration of streptozocin to a patient with advanced ovarian carcinoma. The patient's tumor was rapidly consuming glucose, resulting in a peritoneal fluid glucose < 10 mg/100 ml.

Following the ip administration of 1 g of streptozocin, peripheral blood and peritoneal samples were obtained at various time points up to 5 hours. The EDTA-anticoagulated samples were immediately spun at 4000 rpms for 90 seconds to obtain plasma. One milliliter of plasma or peritoneal fluid was added to 0.25 ml of 3 N perchloric acid and kept at 0°C. The samples were then spun at $2700 \times g$ for 10 minutes at 4°C. The level of streptozocin in the supernatants was determined by the method of Forist (3).

Prior to the administration of streptozocin, 100 ml of peritoneal fluid was removed, and cell-free fluid was obtained by centrifuging at $1000 \times g$ for 10 minutes at 22°C. The half-life of streptozocin in vitro was determined by incubating streptozocin at a concentration of 300 μg/ml in the patient's peritoneal fluid with or without cells at 37°C.

CASE REPORT

A 54-year-old female presented with stage II moderately differentiated ovarian serous cystadenocarcinoma completely resected. Following surgery, the patient was given adjuvant chemotherapy with cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone. Six months after her initial surgery, she developed an abdominal mass and tense ascites, which at a second laparotomy proved to be unresectable recurrent ovarian carcinoma. She was then treated with hexamethylmelamine, cyclophosphamide, doxorubicin, and cisplatin. She improved during this therapy but never obtained a partial response. Within 5 months she demonstrated progressive disease, with an increase in the number of paracenteses required and a declining performance status. She was then treated ip with doxorubicin and cisplatin without any evidence of response.

The patient's peritoneal glucose was always < 10 mg/100 ml (except transient elevations following the ip doxorubicin and cisplatin). Cultures for tuberculosis, fungus, and bacteria were always negative, and cytology was positive. Since it appeared that the tumor was avidly consuming glucose, a course of ip streptozocin was given. The patient received 1 g of streptozocin ip six times within a 3-week period. There was no nausea, vomiting, or evidence of renal dysfunction following this chemotherapy.

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[1] Supported in part by Public Health Service grant CA-28984 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; by a grant from the Cancer Society of Quebec; and by a contribution from Mr. Tibor Schiff.

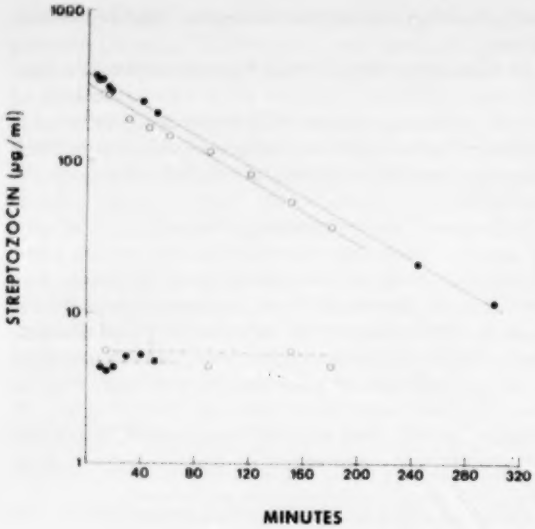

FIGURE 1.—Plasma (broken lines) and peritoneal (solid lines) streptozocin levels following the initial ip administration of 1 g of streptozocin (closed circles) and the 2nd administration 5 days later (open circles).

Serial serum and peritoneal amylases were normal. Streptozocin levels in the plasma and peritoneal fluid were measured on two occasions. The patient's disease continued to progress, and she died. Permission for an autopsy was not obtained.

Peritoneal and plasma streptozocin levels were determined as described (fig 1). There was minimal leakage of streptozocin into the systemic circulation. The half-life of streptozocin in the peritoneal fluid was 61 ± 0.5 minutes (r correlation > 0.9[illegible]), with a volume of distribution of 3 ± 0.4 liters. The half-life of streptozocin in the patient's peritoneal fluid with or without cells in vitro at 37°C was 42 ± 10.4 minutes. The in vitro half-life of streptozocin was shorter than the in vivo half-life.

DISCUSSION

The patient tolerated several ip doses of streptozocin without nausea, vomiting, or evidence of renal dysfunction. The patient did not respond to the therapy, but dose-limiting toxicity was not reached. The pharmacokinetic studies demonstrate that streptozocin was largely confined to the intraperitoneal cavity. The volume of distribution (3 liters) is approximately equivalent to the volume of ascitic fluid present. The shorter in vitro half-life of streptozocin as compared to the in vivo intraperitoneal half-life also suggests that there was no significant absorption into the systemic circulation, since significant absorption into the systemic circulation should have reduced the in vivo intraperitoneal half-life.

Ovarian carcinoma is often limited to the abdomen. In this preliminary report, streptozocin administered ip did not have any significant toxicity and remained largely intraperitoneal. Perhaps the chloroethylnitrosoureas (eg, carmustine) may share the same pharmacokinetics as streptozocin, ie, remain largely intraperitoneal following ip administration. Since all the nitrosoureas have a very short half-life in serum (usually ≤ 60 minutes), they may prove particularly useful for ip therapy, resulting in a very high local concentration without significant systemic toxicity (4). Further studies with nitrosoureas administered ip to patients with malignancies confined to the peritoneum will be necessary to determine if this approach will be useful.

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Lawrence C. Panasci
Violetta Skalski
Judith St-Germain
Philip Lazarus
Michael Shinder
Richard Margolese[2]

McGill Cancer Center
Jewish General Hospital
3755 Chemin de la Côte Ste-Catherine
Montreal, Quebec, Canada
HET 1E2

March 18, 1982

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[2] We thank Miss Genevieve Leclercq for preparation of the manuscript.

Trial of AMSA in Acute Leukemia[1,2]

The acridine derivative AMSA has had antitumor activity in preclinical systems and has demonstrated

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[1] Supported by the Co-operative Cancer Treatment Research Program, a project of the Pan American Health Organization and the National Cancer Institute, National Institutes of Health, Department of Health and Human Services (contract N01-CO-65332).

[2] Presented at the 18th Annual Meeting of the American Society of Clinical Oncology, April 30–May 2, 1981.

antileukemic activity in humans (1). The latter has been achieved with total drug doses > 360 mg/m^2 delivered in 3–5 days (2–6). We wish to comment on a trial done at the National Academy of Medicine in Buenos Aires, Argentina, that adds to the present experience with AMSA, especially in lymphoblastic leukemia. In a recent study, we administered AMSA to 17 patients with heavily pretreated acute leukemia. There were ten patients with lymphoblastic leukemia (ALL), six with myelogenous leukemia (AML), and one with accelerated (blast) phase chronic myelogenous leukemia (BC-CML). All had been pretreated with daunorubicin. The AMSA dose was 75–90 mg/m^2/day iv × 7. The median age of the patients with ALL was 15 years (range, 6–36), and eight of them were between 10 and 26 years old. Of these ten patients with ALL, two had bone marrow responses to AMSA after one drug course: one achieved a complete response (CR) lasting 49 days and the other achieved a partial response (PR) lasting 21 days. Both patients received a second course but had no further improvement. A third patient had a 70% reduction in spleen size after two courses but no change in bone marrow. Four of the remaining patients achieved aplasia but showed only return of leukemia cells. All patients showed transient reduction or clearing of circulating blast cells from peripheral blood. Among the other six patients, one with AML had a complete bone marrow response lasting > 280 days and the others showed only transient reduction in circulating blast cells.

The toxic effects of AMSA included nausea and vomiting, alopecia, local pain, phlebitis, and transient skin rash. Of particular note was the occurrence of jaundice in four patients. One 10-year-old girl developed liver failure and died in hepatic coma 2 weeks after the first AMSA cycle was completed. The serum transaminases were repeatedly normal, but the total bilirubin rose to 21 mg/dl prior to death. She had a positive hepatitis B antigen titer but no history of overt hepatitis. She was not taking excessive doses of acetaminophen or other drugs.

These results show clinical activity of AMSA in ALL and compare favorably with results from other centers. Legha et al (2) achieved 13 CRs and six PRs in 56 patients, but none of these occurred in 13 adult patients with ALL. Arlin et al (3) achieved three CRs in ten patients with ALL and in three of 17 with acute nonlymphocytic leukemia (ANLL), but achieved none in four patients with BC-CML.

Van Echo et al (4) achieved four CRs in 17 patients with ANLL and one PR in five with ALL, but no response in five with BC-CML. Land et al (5) achieved six CRs and three PRs in 35 children with leukemia, including three CRs and two PRs in 21 patients with ALL. One additional report (6) showed two CRs in nine children with advanced lymphoblastic leukemia.

Our data combined with those in the other reports indicate a total of 13 observed CRs and PRs in 68 patients of all ages with ALL. The drug deserves further trial as part of induction and consolidation regimens for lymphoblastic leukemia. However, the potential of AMSA to produce jaundice and liver dysfunction needs emphasis, and patients with preexisting liver disease or who are receiving other hepatotoxic drugs such as acetaminophen should be watched carefully while receiving AMSA therapy.

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Juan C. Dupont
Guy E. Garay
Cristina Scaglione
Santiago Pavlovsky

Oncohematology Department
Instituto de Investigaciones
Hematologicas
Academia Nacional de Medicina
Buenos Aires, Argentina

Paul V. Woolley[3,*]

Division of Medical Oncology
Georgetown University Hospital
and Vincent T. Lombardi Cancer
Research Center
Washington, DC 20007

March 25, 1982

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[3] We acknowledge the participation of Ms. Ana M. Tormena and Ms. Estela Koval in the clinical care of the patients.

**Reprint requests to:* Paul V. Woolley, MD, Rm 2100 West, Georgetown University Hospital, 3800 Reservoir Rd, NW, Washington, DC 20007.

ANNOUNCEMENTS

Approaches to the Management of Pain

Oakland, California, September 9, 1982

This 1-day conference will deal with the multidisciplinary approach to the management of pain. Cosponsoring organizations are the Bay Area Tumor Institute and the East Bay Cancer Program. The program will be held in Goodman's Hall, 10 Jack London Square.

For further information, contact: Despina C. M. Johnson, MPH, Program Manager, 2844 Summit St, Suite 204, Oakland, CA 94609 (telephone: 415-465-8570).

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. Although teaching will be primarily provided through lectures, panel discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00.

For further information, contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Paraneoplasia: Basic Concepts and Clinical Aspects

Brussels, Belgium, November 5–6, 1982

This 2-day symposium presented by the European Organization for Research on Treatment of Cancer will focus on endocrine paraneoplasia, neurologic paraneoplasia, coagulopathies, and nonhormonal tumor markers. The Chairmen are J. Waldenström and H. J. Tagnon.

In addition, free communications of 10 minutes each will be accepted. An abstract must be sent to the address below before September 30, 1982. Seating is limited and preregistration is recommended.

For further information, contact: Dr. M. Staquet, Director, EORTC Data Center, rue Héger-Bordet 1, 1000 Brussels, Belgium. Telephone: (2) 538.57.90, Telex: 22773.

Gastrointestinal Tumor Study Group

The Gastrointestinal Tumor Study Group (GITSG), an NCI-funded, multidisciplinary clinical trials group, invites institutions and individuals who are forming a Community Clinical Oncology Program (CCOP) to participate in GITSG colon, rectal, gastric, and pancreatic studies. *For information, please contact* Philip S. Schein, MD, c/o ECTO Operations Office, Suite 214, 11325 Seven Locks Rd, Potomac, MD 20854. Telephone: 301/299-8655.

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NIH Publication No. 82-22

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1982

cancer treatment reports

August 1982
Volume 66
Number 8
Pages 1601-1686

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

HE 20. 3160:

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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

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1685 ANNOUNCEMENTS

Anticipatory Vomiting in Women Receiving Cyclophosphamide, Methotrexate, and 5-FU (CMF) Adjuvant Chemotherapy for Breast Carcinoma[1,2]

Patti M. Wilcox,[3,*] **John H. Fetting,**[3] **Kathleen M. Nettesheim,**[4] **and Martin D. Abeloff**[3]

To determine the incidence of anticipatory vomiting (AV) and postchemotherapy nausea and vomiting (PCNV) in women receiving cyclophosphamide, methotrexate, and 5-FU (CMF) adjuvant chemotherapy for breast carcinoma, we studied 52 women randomized to two regimens (standard-dose and low-dose) of CMF. Charts were reviewed for the cycle of onset of AV and PCNV, the severity of PCNV, and relationships of these syndromes to CMF dose and protocol compliance. Among the 52 patients, AV occurred in 17 (33%), while PCNV was experienced by 46 (88%). Severe PCNV (defined as uncontrolled nausea and/or vomiting interfering with performance of daily activities) occurred in 22 of 52 (42%) women. Eighteen of 23 (78%) women receiving standard-dose CMF experienced severe PCNV, and 13 of these had AV. Patients in whom severe PCNV began before cycle 4 were more likely to develop AV than women in whom PCNV began later ($P < 0.01$). Ten of 52 (19%) patients discontinued CMF adjuvant chemotherapy because of nausea and vomiting; seven of the ten (70%) were receiving standard-dose CMF and seven had experienced AV. This study demonstrates that both AV and PCNV are significant toxic effects that not only affect the quality of life of a woman receiving CMF chemotherapy for breast cancer but also limit the ability of the clinician to provide maximum therapy to women at high risk of recurrence of breast carcinoma. [Cancer Treat Rep 66:1601–1604, 1982]

Nausea and vomiting following the administration of cytotoxic agents presumably results from an interaction between an antineoplastic agent and some station along the afferent and efferent reflex arc including the gastrointestinal tract, spinal cord, and brain stem (1,2). Such postchemotherapy nausea and vomiting (PCNV) is a dose-limiting toxic effect of chemotherapeutic agents (3). Recently, another chemotherapy-related nausea and vomiting syndrome, called anticipatory nausea and vomiting, has been reported (4,5). This syndrome is defined as nausea and vomiting prior to, and in anticipation of, chemotherapy administration; its pathogenesis is unknown.

This paper reports the incidence and time of onset of anticipatory vomiting (AV) and PCNV in a series of women with breast carcinoma treated with adjuvant chemotherapy and relates the development of AV to the severity of PCNV. Protocol compliance by the study population affected by AV and PCNV was also analyzed.

MATERIALS AND METHODS

Population Characteristics

The study consisted of a retrospective chart review of 52 patients treated at The Johns Hopkins Oncology Center (JHOC). The patients, a subgroup of a larger community cancer network trial, had undergone radical or modified radical mastectomy for stage II or III breast carcinoma. They were randomly assigned to two regimens of cyclophosphamide, methotrexate, and 5-FU (CMF) given every 28 days for 24 cycles. The purpose of the chemotherapy study was to compare the therapeutic efficacy and toxicity of two CMF regimens: *standard-dose CMF*—100 mg/m^2 of cyclophosphamide orally on Days 1–14, 40 mg/m^2 of methotrexate iv on Days 1 and 8, and 600 mg/m^2 of 5-FU iv on Days 1 and 8; and *low-dose CMF*—40 mg/m^2 of cyclophosphamide orally on Days 1–14, 15 mg/m^2 of methotrexate iv on Days 1 and 8, and 350 mg/m^2 of 5-FU iv on Days 1 and 8. Of the 52 JHOC participants, 23 (44%) were randomized to standard-dose CMF and 29 (56%) to low-dose CMF. No patients received radiotherapy or hormonal therapy during this study.

Twenty-four of the women (46%) were premenopausal and 28 (54%) were postmenopausal, with ages ranging from 29 to 66 years. The 52 JHOC patients did not vary significantly from the total population of 93 women in the community cancer network trial in terms of menopausal status, number of axillary nodes involved with tumor, tumor size, or CMF dose randomization. The study pop-

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[1] Received Nov 30, 1981; accepted Apr 14, 1982.

[2] Supported in part by Public Health Service grant CA-17448 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Nursing, The Johns Hopkins Hospital *(P. M. Wilcox)* and The Johns Hopkins University School of Medicine *(J. H. Fetting,* and *M. D. Abeloff)*, The Johns Hopkins Oncology Center, Baltimore, MD.

[4] Department of Oncology, Ambulatory Care Center, Mount Sinai Medical Center, Milwaukee, WI.

[*] *Reprint requests to:* Patti M. Wilcox, RN, The Johns Hopkins Oncology Center, B-111, Outpatient Department, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—AV and CMF dose

Group	No. (%) of patients		
	Total	Without AV	With AV
Standard-dose CMF	22*	9 (41)	13 (59)
Low-dose CMF	29	25 (86)	4 (14)†
Total	51*	34 (67)	17 (33)

*1 patient receiving standard-dose CMF who refused treatment because of severe PCNV after cycle 1, Day 1, could not be categorized as having or not having AV.

†$P < 0.01$.

ulation did differ from the community program group in that the 41 women followed in the community were seen by a physician alone, while only 17 of the 52 JHOC patients (33%) were seen by a physician alone. The remaining 35 women (67%) in the JHOC group were followed in joint practice by a physician and a nurse practitioner (P. M. Wilcox). Thus, many of the JHOC participants were evaluated by two health care providers during their course of therapy. The preliminary results of the entire community cancer network trial are reported elsewhere (6).

Assessment of Nausea and Vomiting

Based on clinical reports, we defined AV as vomiting prior to the administration of CMF adjuvant chemotherapy without a known cause for vomiting (eg, concurrent illness or evidence of metastatic disease). As part of the goal to evaluate CMF toxicity, the principal investigator (M. D. Abeloff) developed guidelines for rating severity of PCNV and for modifying CMF dose accordingly. The grading of PCNV included none, moderate (requiring antiemetics but controllable), and severe (uncontrolled, interfering with performance of daily activities). Severe PCNV required a 50% reduction of CMF dose per protocol. The chart review of the 52 cases was accomplished by recording the date(s) and grade of PCNV and the date(s) of AV from the clinician's narrative record and the computerized protocol symptom list. We related the severity of the PCNV to the development of AV and analyzed the impact of these nausea and vomiting syndromes (PCNV and AV) on protocol compliance. Statistical relationships were calculated using the standard chi-square and two-tailed T tests.

RESULTS

AV occurred in 17 of 52 women (33%). Table 1 demonstrates the relationship between AV and the dose levels of CMF. AV occurred more frequently in women receiving standard-dose CMF than in patients receiving low-dose CMF ($P < 0.01$). PCNV was experienced by 46 of the 52 women (88.5%) in the study. Table 2 shows the incidence of PCNV in relationship to the dose levels of CMF. There is a statistically significant relationship between the incidence of severe PCNV and the higher CMF dose ($P < 0.001$).

The relationship between the severity of PCNV and AV is shown in table 3. All 17 women who developed AV experienced prior PCNV, and 13 of the 17 (76.5%) reported severe PCNV. AV occurred more frequently in women with severe PCNV than in those with moderate PCNV ($P < 0.001$).

The cycle of onset of PCNV was similar for both those women who experienced AV (mean cycle, 2.17; median cycle, 1) and those who did not experience AV (mean cycle, 2.50; median cycle, 2). However, the cycle of onset of severe PCNV varied considerably between the two groups: patients with AV had a mean cycle of onset of 3.46 (median cycle, 3: SE, 0.82) while patients without AV had a mean cycle of onset of 8.62 (median cycle, 9; SE, 2.1). These data indicate that patients who developed severe PCNV early in their course of treatment (before cycle 4) were more likely to eventually develop AV, compared to patients who developed severe PCNV later in their therapy ($P < 0.01$).

Twenty-one of 22 patients who experienced severe PCNV had one or more 50% reductions in prescribed CMF dose. One patient discontinued CMF therapy after

TABLE 2.—PCNV and CMF dose

Group	No. (%) of patients			
	Total	No PCNV	Moderate PCNV	Severe PCNV
Standard-dose CMF	23	1 (4)	4 (17)	18 (78)
Low-dose CMF	29	5 (17)	20 (69)	4 (14)*
Total	52	6 (12)	24 (46)	22 (42)

*$P < 0.001$.

TABLE 3.—AV and PCNV severity

	No. (%) of patients			
Group	Total	No PCNV	Moderate PCNV	Severe PCNV
With AV	17	0	4 (23.5)	13 (76.5)
Without AV	34	6 (17.6)	20 (58.8)	8 (23.5)*
Total	51†	6 (11.8)	24 (47.1)	21 (41.2)

*$P < 0.001$.

†1 patient who refused treatment because of severe PCNV after cycle 1, Day 1, could not be categorized as having or not having AV.

cycle 1, Day 1, and thus is not recorded as having a dose reduction. Of the 21 women who received protocol dose reductions, 17 (81%) were receiving standard-dose CMF, while only four (19%) were receiving low-dose therapy. Fourteen of 52 patients (27%) discontinued CMF adjuvant chemotherapy before cycle 24; ten of the 14 (71%) who discontinued therapy cited nausea and vomiting as the major reason for stopping treatment. All ten patients who discontinued therapy because of nausea and vomiting had experienced severe PCNV: seven (70%) had received standard-dose CMF and three (30%) had received low-dose CMF. Seven of the ten women (70%) who discontinued CMF adjuvant chemotherapy due to nausea and vomiting had experienced AV. Four of 14 women who discontinued therapy did so for reasons unrelated to nausea and vomiting.

DISCUSSION

In this study of 52 women randomized between two regimens of CMF adjuvant chemotherapy for stage II or III breast carcinoma, 17 (33%) developed AV. AV occurred more frequently in patients with severe PCNV and in patients receiving the higher dose of CMF. Ten of 14 women who discontinued treatment did so because of nausea and vomiting and seven of these ten had experienced AV.

Since patients in the community cancer network were not included in our chart review, the AV incidence of 33% in the JHOC population may not accurately reflect the incidence in the larger group. The retrospective nature of this study limited the observed rate of AV to that recorded by the health care provider, which in turn limits the accurate assessment of the incidence of AV. On the other hand, the reliability and validity of the retrospectively derived incidence figure may be enhanced by the fact that two thirds of the sample population were followed by one clinician (P. M. Wilcox). These data should not be generalized to larger populations without further study, because the incidence of AV may differ for patients with breast cancer treated with similar regimens in different settings or treated with different regimens.

There was a significant relationship between both higher doses of CMF adjuvant chemotherapy and severe PCNV and the development of AV. Since no patient developed AV without first developing PCNV, it seems likely that the relationship between the higher dose of CMF and AV is mediated through the severe PCNV caused by that regimen. The data regarding the time of onset of PCNV show that, while all patients who developed mild PCNV did so at approximately the same time, women who developed severe PCNV before cycle 4 were at a significantly greater risk for developing AV.

The pathogenesis of AV is unknown. However, since AV did not develop in any woman who did not first have at least moderate PCNV, AV may be a Pavlovian conditioned response (7,8). Reports in the literature suggest that systematic desensitization and relaxation techniques have been helpful for some patients with AV (9,10). Since AV and severe PCNV resulted in the reduction of CMF doses and accounted for 71% of the treatment dropouts, these syndromes may indirectly affect disease-free interval and survival. Bonadonna and Valagussa (11) have suggested that the length of the disease-free interval and survival are related to the total dose of CMF administered.

In our population, AV and PCNV were significant problems that warrant better understanding and more effective interventions. Studies that prospectively assess nausea and vomiting syndromes are indicated, as are studies that use the expertise of multidisciplinary investigators in planning, implementing, and evaluating interventions for amelioration and/or prevention of AV and PCNV.

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Combined Doxorubicin and Radiation Therapy in Malignant Pleural Mesothelioma[1,2]

Clive Sinoff, Geoffrey Falkson,* A. G. Sandison, G. De Mûelenaere [3]

Ten patients with histologically confirmed inoperable malignant mesothelioma of the pleura were treated with doxorubicin and fractionated radiotherapy courses. Three patients derived significant clinical benefit from this treatment, although only one of the three had measurable tumor shrinkage that could be defined as partial response. Two of the ten patients showed only progressive disease, while the remaining five showed disease stabilization for 30–100 weeks. The treatment was subjectively well-tolerated and hematopoietic toxicity was acceptable. Radiation pneumonitis did not occur. Two of the four patients who lived ≥ 94 weeks developed fibrosis of the irradiated hemithorax. The median survival time for all patients was 46 weeks. Although the combined treatment could be given with acceptable toxic effects and although four patients benefited from it, the best objective assessment, namely, survival time, did not appear to be adequately influenced to justify an extension of this series. [Cancer Treat Rep 66:1605–1607, 1982]

Long-term survival of patients with malignant mesothelioma after radiation therapy alone has been reported in isolated instances (1,2), but the usual result is disappointing (1–3). The best results reported in the treatment of this disease have been with the combined use of procarbazine and radiation therapy (3). In that study, various irradiation schedules were used. The best response was observed among 11 patients receiving 1800–2000 rad in 2 weeks and in 12 patients treated with < 1600 rad; both treatments were repeated every 2 months. The total dose of radiation is difficult to interpret because up to eight courses of treatment were given in up to 2 years.

Doxorubicin as a single agent showed promising activity in early studies (4,5). In an Eastern Cooperative Oncology Group (ECOG) study of 51 patients, doxorubicin showed no important activity (6). The present study was undertaken to assess whether it is possible to use the multiple fractionated irradiation technique developed in Pretoria (3,7–10) together with full therapeutic doses of doxorubicin, without causing unacceptable toxicity. It was hoped that the therapeutic benefit that might be obtained from doxorubicin could be enhanced by the addition of radiation therapy. The advantage of irradiation fractionation is that a large dose of radiation would not be given to nonresponders, and responders could be carefully monitored while the cumulative dose of radiation increased.

MATERIALS AND METHODS

Six men and four women ranging in age from 34 to 69 years (median, 56) with advanced mesothelioma confined to one hemithorax were treated during 1979 and 1980. All patients had inoperable, histologically confirmed malignant pleural mesothelioma. The ECOG performance status (PS)[4] was 3 in one patient, 2 in two patients, 1 in six patients, and 0 in the remaining patient. Thoracotomy was performed in all ten patients. Resection was attemped in one patient without success. In the remaining nine patients, disease was too advanced to permit resection, although resection had been considered in all patients. An estimate of the degree of pleural involvement (from fine nodulation to massive tumor in the hemithorax) obtained at thoracotomy was combined with the radiologic findings to assess the tumor load in each patient.

It was not possible to estimate all areas of pleural thickening in the presence of massive pleural effusion. Patients were evaluated following thoracocentesis (table 1). Extent of disease was defined as follows: *moderate*—loss of < 1/3 of radiographically visible lung tissue (extent I); *marked*—loss of 1/3–2/3 of radiographically visible lung tissue (extent II); and *extensive*—loss of 2/3 of radiographically visible lung tissue (extent III). This classification was used irrespective of whether the lung was compressed by tumor, infiltrated by tumor, or considered

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[1] Received Nov 4, 1981; revised Apr 22, 1982; accepted May 10, 1982.

[2] Supported in part by a grant from the National Cancer Association of South Africa.

[3] Departments of Chemotherapy and Radiotherapy, University of Pretoria, Republic of South Africa.

[4] ECOG PS criteria: 0 = normal activity; 1 = symptoms but ambulatory; 2 = in bed < 50% of time; 3 = in bed > 50% of time; and 4 = 100% bedridden.

*Reprint requests to: Geoffrey Falkson, MD, Department of Cancer Chemotherapy, H. F. Verwoerd Hospital and University of Pretoria, Private Bag X169, Pretoria, 0001, Republic of South Africa.

TABLE 1.—Doxorubicin and radiotherapy in treatment of malignant pleural mesothelioma

Patient No.	Age (yrs)*	Sex	PS†	Extent of disease†	Time to treatment failure (wks)*	Survival (wks)*	Comments
1	37	M	0	II	32	44	Reduction of pleural thickening (> 50%); clearing of pleural effusion; and weight gain
2	49	F	1	I	100	106	Fibrosis of irradiated hemithorax; improvement in PS; and reduction in thoracic pain
3	69	F	2	II	82	96	Fibrosis of irradiated hemithorax; improvement in PS; reduction in chest pain and dyspnea
4	34	F	2	III	32	45	Improvement in PS; and reduction in pleural pain, dyspnea, and cough
5	56	F	1	II	90	94	No change
6	64	M	1	I	52	108	No change
7	44	M	1	III	30	48	No change
8	68	M	1	II	30	44	No change
9	56	M	3	II	13	28	Disease progression only
10	69	M	1	II	9	19	Disease progression only

*Median age, 56 yrs; median survival, 46 wks ($10^{1}/_{2}$ mos); median time to treatment failure, 32 wks.

†*See* text for definitions.

to contain metastatic mesothelioma within the lung tissue. A separate estimation for pericardial involvement was not possible, although the pericardium was considered to be involved in all ten patients. None of the patients had received chemotherapy or radiotherapy prior to entry in this study. All patients were directly referred from thoracic surgery. Renal, hepatic, and cardiac functions were normal. Because of the difficulties in objectively evaluating response in patients with malignant pleural mesothelioma, survival was the primary end point for evaluation in this study. Response was measured clinically, with emphasis on respiratory reserve, and radiologically. Special attention was paid to change in PS and to subjective change.

Doxorubicin was given at a dose of 60 mg/m^2 iv every 3 weeks. The dose was decreased by 50% if the wbc count on Day 1 of each treatment cycle was < 4000 but > 3000/mm^3 and/or the platelet count was < 100,000 but > 75,000/mm^3. The cycle was postponed until recovery when the wbc count was < 3000/mm^3 and the platelet count was < 75,000/mm^3. The maximum cumulative dose of doxorubicin allowed was 500 mg/m^2.

Radiotherapy was given concomitantly with the first, third, fifth, and seventh doses of doxorubicin. Cobalt-60 radiation was used, giving a tumor dose of 200 rad/day, to a total dose of 1000 rad in 5 days, commencing on Monday and ending on Friday. Parallel opposing fields to include the involved side of the hemithorax plus one half of the mediastinum were used. The field size varied from 19 × 19 cm to 25.5 × 27 cm. No correction was made for lung tissue. A maximum of four such courses of radiotherapy was planned for each patient.

RESULTS

Seven of the ten patients received the planned maximum treatment (four courses of radiotherapy and a cumulative doxorubicin dose of 500 mg/m^2). Three patients received less than the planned maximum dose because of disease progression. One patient received the full four courses of irradiation but only 530 mg of doxorubicin, one received three courses of radiation therapy and 400 mg of doxorubicin, and one received only one course of irradiation but 340 mg of doxorubicin.

Toxicity

Leukopenia < 4000 but > 3000 cells/mm^3 was documented in four patients. Leukopenia < 3000 but > 2000 cells/mm^3 was documented in three patients. No patient had a wbc count < 2000/mm^3. The seven patients with mild or moderate leukopenia required dose modification (reduction in dose or postponement). Thrombocytopenia requiring dose modification did not occur. Nausea and vomiting occurred in six patients but was never severe. One patient developed cardiac failure 14 months after completion of therapy. This was a preterminal event but was possibly related to treatment; it occurred $93^{1}/_{2}$ weeks after the start of treatment. Two patients who received the full planned dose of radiation and doxorubicin developed total fibrosis of the irradiated hemithorax. This was the expected reaction to the irradiation. None of the patients had acute radiation pneumonitis or esophagitis.

Response

One patient had a partial response as judged by a definite reduction in pleural thickening (> 50%) and pleural fluid. This patient's PS remained 0, and no new lesions developed during the 32 weeks before disease progression occurred.

Two female patients, both of whom developed fibrosis of the irradiated hemithorax, were considered to be objectively improved and had marked subjective improvement. They had a decrease in respiratory distress with definite subjective improvement, showed improvement in PS, and were able to return to work. These patients survived 106 and 96 weeks. Neither patient had total involvement of the hemithorax at the start of treatment, as judged by the above criteria. It was not possible to distinguish clinically between fibrosis and tumor. Neither patient showed disease progression or extension. Another patient had subjective improvement in PS, and the tumor remained static for 8 months.

Four patients had no change in tumor status for 90, 52, 30, and 30 weeks. In these patients the PS and subjective state remained constant. The remaining two patients showed measurable disease progression despite treatment.

The time to treatment failure varied from 9 to 100 weeks (median, 32). Survival ranged from 19 weeks ($4^{1}/_{2}$ months) to 108 weeks (26 months), with a median of 46 weeks ($10^{1}/_{2}$ months).

DISCUSSION

The combined treatment was well-tolerated. Only one of the ten patients developed overt cardiac failure and this occurred at a time when the patient had progresssive disease. Hematologic and gastrointestinal toxic effects were acceptable. The absence of symptomatic pneumonitis is most probably due to the prolonged time span ($4^{1}/_{2}$ months) over which the irradiation was administered. Such fractionation has far less biologic effect than the same dose given consecutively in a compressed time span. The incidence of symptomatic pneumonitis in patients receiving one 800-rad prophylactic lung radiation treatment for osteogenic sarcoma is low. With a dose of 1000 rad over 5 days, symptomatic pneumonitis would not be expected if irradiation had been the only treatment. The total dose of doxorubicin ranged from 340 to 800 mg, with a median of 615 mg over 22 weeks. This is considered to be an adequate dose of doxorubicin. It is improbable that a higher dose of radiation would be tolerated.

Partial response as documented by strict tumor measurement occurred in only one patient, but an objective improvement occurred in another two patients. The disease progressed relentlessly in only two patients.

Survival ranged from 19 to 108 weeks (median, 46: $10^{1}/_{2}$ months). This is comparable to the results of Chahinian and Holland (11) using doxorubicin and nonfractionated irradiation. Similar survival times have been reported in patients with pleural mesothelioma treated with a variety of single agents or combinations of agents or radiation therapy alone (12–17). Because the major measurable end point of the present study is survival, the most likely explanation for the lack of benefit is that neither doxorubicin nor radiotherapy alone is sufficiently effective in the treatment of this disease to make the combined modality approach worthwhile. The treatment combination was relatively well-tolerated; however, this is not sufficient motivation to extend the study.

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Upper Hemibody and Local Chest Irradiation as Consolidation Following Response to High-Dose Induction Chemotherapy for Small Cell Bronchogenic Carcinoma—A Pilot Study[1,2]

Bernard A. Mason,* Melvyn P. Richter, Robert B. Catalano, and Richard B. Creech[3,4]

Encouraging results of the combination of upper hemibody irradiation (UHBI) and local chest irradiation (LCI) combined with standard-dose chemotherapy in patients with extensive small cell bronchogenic carcinoma led us to a second pilot study utilizing the same radiation program combined with high-dose induction chemotherapy. Fourteen patients with small cell bronchogenic carcinoma, five with extensive disease and nine with localized disease, were treated with cyclophosphamide (1.5 g/m² iv, Days 1 and 22), lomustine (70 mg/m² orally, Day 1), and methotrexate (15 mg/m² twice weekly during Weeks 2, 3, 5, and 6). UHBI (600 rads) was given during Week 6 in a single dose and LCI was given during Week 7 (2000 rads/five fractions) to the tumor and mediastinum. Maintenance chemotherapy began in Week 12 with cyclophosphamide (700 mg/m² iv every 3 weeks) and lomustine (70 mg/m² orally every 6 weeks). Twelve patients were evaluable for response and toxicity (eight with limited disease). There were three complete responses and seven partial responses after induction chemotherapy. After completion of the consolidation radiation therapy, all 12 patients had a response: six complete responses and six partial responses. Acute toxic effects included nausea and vomiting in eight patients, fever in five, and hypotension and angina in one. Subacute toxic effects included nausea, vomiting, and dehydration in two patients who required hospitalization, prolonged aplasia in one, and reversible radiation esophagitis in three. Three patients had radiation pneumonitis including one with bilateral diffuse disease that led to death from respiratory failure. Only two of 12 patients received their maintenance therapy on schedule. Treatment failures occurred within the LCI field in seven patients and in distant metastatic sites in six. The median time to first relapse was 7 months and the median survival was 9 months. Because of toxicity, treatment delays, and poor survival in this group of patients, we cannot recommend this combined modality approach. [Cancer Treat Rep 66:1609–1612, 1982]

Progress in the treatment of small cell bronchogenic carcinoma (SCBC) has reached a plateau (1–3). Aggressive combination chemotherapy programs have resulted in high response rates and in an increased percent of complete remissions. Survival of all patients has increased about fourfold to fivefold, with the median survival times increasing from 2.5 to about 12 months with modern therapy (1,2). However, despite the initial responsiveness of their tumors to chemotherapy, patients with disease remission have a pattern of continuous relapse and ultimately die of progressive disease. Innovative efforts to prevent relapses in general or in specific sites, particularly in complete responders, have included consolidative chest irradiation in patients with limited disease (1), prophylactic brain irradiation (4), cyclic alternating non-cross-resistant combination chemotherapy programs (5), intensive high-dose chemotherapy (3), and the addition of thymosin fraction V (6). None of these approaches has resulted in unqualified success in solving the problem of continuous relapse.

Recently, a pilot study was performed of upper hemibody irradiation (UHBI) and local chest irradiation (LCI) combined with standard doses of cyclophosphamide and lomustine in patients with extensive SCBC (7). The UHBI was intended as a form of systemic therapy that could substitute for a cycle of chemotherapy and possibly consolidate the treatment of disease in the primary and major metastatic sites (mediastinum, liver, upper abdominal

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[1] Received Nov 24, 1981; revised Mar 31, 1982; accepted May 10, 1982.

[2] Dr. Mason is a Junior Faculty Clinical Fellow of the American Cancer Society.

[3] Departments of Medicine and Radiotherapy, The American Oncologic Hospital, The Fox Chase Cancer Center, and the University of Pennsylvania School of Medicine, Philadelphia.

[4] We thank Drs. Harry Katz, Richard Cumberlin, Barbara Lambert, and John Young for their help and participation in this study, and Ms. Hilary Daniels for preparing the manuscript.

**Reprint requests to:* Bernard A. Mason, MD, The Graduate Hospital, Pepper Pavilion, Suite 502, 19th and Lombard Sts, Philadelphia, PA 19146.

lymph nodes, adrenals, bones, and brain). The toxicity of this combined modality approach was acceptable, and the response rate and median survival were almost identical to those of an historical control group that had received chemotherapy alone. The encouraging results of this feasibility study led us to a second pilot study utilizing the same radiation program combined with high-dose induction chemotherapy.

MATERIALS AND METHODS

Fourteen patients with SCBC were entered in this combined modality study. Five patients had extensive disease and nine had localized chest disease. The median performance status (PS) was 1 and the mean PS was 1.2 (Eastern Cooperative Oncology Group PS 0–4). Two patients (one with extensive and one with localized disease) did not receive the consolidative radiation therapy program: the patient with local disease died of sepsis resulting from leukopenia during high-dose induction chemotherapy and the other patient with a low PS after the induction chemotherapy was not eligible for the UHBI. The remaining 12 patients who received the entire induction treatment program were evaluable for toxic effects and response. Their median age was 60 years (oldest patient, 69 years old). All patients had adequate peripheral blood determinations (hemoglobin > 10 g/100 ml, wbc count $> 4000/\text{mm}^3$, and platelet count $> 100{,}000/\text{mm}^3$), renal function (BUN < 20 mg/100 ml), and hepatic function (bilirubin less than two times normal).

Induction chemotherapy consisted of cyclophosphamide (1.5 g/m^2 iv on Days 1 and 22), lomustine (70 mg/m^2 orally on Day 1), and methotrexate (15 mg/m^2 orally twice weekly during Weeks 2, 3, 5, and 6). There was no dose modification for cytopenias during the 6-week induction period. Patients were treated with consolidation therapy between the 7th and 9th weeks. Maintenance chemotherapy was scheduled to begin in Week 12 with cyclophosphamide (700 mg/m^2 iv every 3 weeks) and lomustine (70 mg/m^2 orally every 6 weeks) until tumor progression.

Consolidation radiation therapy consisted of UHBI and LCI. The UHBI was delivered as a single dose of 600 rads to the midthickness of the patient above the iliac crest at a rate of 30–40 rads/minute. The technique of irradiation and the premedication program to ameliorate nausea and avoid hypotensive episodes have been published previously (8). One week later, LCI was administered with anterior and posterior portals that were designed to encompass the primary tumor, the mediastinum, and both supraclavicular areas. A posterior spinal cord block was added at 1200 rads to the involved fields to limit the radiation dose to the spinal cord. Thus, the central mediastinal structures received a reduced radiation dose as well. In those patients with disseminated disease involving bone, local radiation therapy (2000 rads in divided fractions) was administered concurrent with the local chest irradiation. The total local dose of 2000 rads was delivered in five fractions of 400 rads each over a period of 1 week.

All patients were followed until their deaths. Responses to and toxic effects of the therapy, as well as the delays in therapy and sites of relapse, were recorded and analyzed. Eastern Cooperative Oncology Group standard criteria for defining response and toxicity were utilized. Limited disease was defined as disease of one hemithorax including the mediastinum but not ipsilateral supraclavicular nodes; extensive disease was defined as disease more advanced than limited disease.

RESULTS

Ten of the 12 evaluable patients responded to the induction chemotherapy: three complete responses (CRs) in patients with limited disease, and seven partial responses (PRs) (three in patients with limited and four in patients with extensive disease).Two patients with limited disease had minor responses to the induction chemotherapy. After completion of the consolidation radiation therapy, all patients achieved a response: six CRs and six PRs. Three patients with a PR after chemotherapy (two with limited and one with extensive disease) converted to CR status and both chemotherapy nonresponders achieved PRs.

As expected, the intensive chemotherapy regimen, which was not modified for blood cell counts during the 6 weeks of induction, was toxic. All patients experienced some degree of pancytopenia although severe leukopenia was usually of short duration. Ten patients developed wbc count nadirs $< 1000/\text{mm}^3$; nadirs for the two remaining patients were 2900 and 3400/mm^3. Seven patients developed platelet count nadirs between 20,000 and 50,000/mm^3, and another had a nadir of 77,000/mm^3. Five of the original 14 patients experienced fever and leukopenia, including one who had salmonellosis, one who had a renal stone and urinary sepsis with *Proteus* organisms, two who required hospitalization for empiric antibiotics, and one who died from sepsis. One patient required platelet transfusions during an episode of thrombocytopenia and occult gastrointestinal blood loss. Five patients experienced mucositis from the methotrexate which was mild and quickly reversible. One patient had hemorrhagic cystitis from his first dose of cyclophosphamide, but recovered completely and received subsequent courses of cyclophosphamide with careful iv hydration.

Acute toxicity of the UHBI included nausea and vomiting in eight patients which began abruptly at the conclusion of the UHBI and was self-limited, and fever between 101°F and 104°F in five patients the night of the UHBI treatment. One patient developed intractable nausea and vomiting and required readmission for iv fluids and supportive care. A final patient with underlying symptomatic coronary artery disease developed severe chest pain and mild hypotension during and immediately following UHBI and required admission to the coronary care unit for preinfarction angina. She recovered completely from this acute episode, but subsequent chronic malaise prevented initiation of maintenance chemotherapy.

Subacute toxic effects resulting from the UHBI plus the LCI (and presumably the substrate of high-dose chemotherapy that preceded the radiation) were observed in nine of 12 patients. Five patients required rehospitalization either during or immediately following the LCI: two had intractable nausea and vomiting; one developed prolonged aplasia; one developed acute fever, malaise, anorexia, weight loss, and leukopenia; and one developed fever, anorexia, pulmonary infiltrates, and hypoxemia. Three patients had self-limited radiation esophagitis; one patient had otitis media. All patients with subacute toxic effects recovered completely, except for the patient who developed prolonged pancytopenia. This patient received UHBI 1 week late due to a wbc count of 2700/mm^3. Following the completion of UHBI and LCI, the wbc and platelet counts ranged between 2000 and 3000 and 30,000 and 80,000/mm^3, respectively, for the next 7 months until her disease recurred.

Late toxic effects from this combined modality induction program were experienced by four patients, including the one patient with pancytopenia (which precluded subsequent maintenance chemotherapy), two patients with localized radiation pneumonitis (both improved with corticosteroid therapy), and one patient with irreversible diffuse bilateral radiation pneumonitis while in clinical CR. This latter patient underwent open-lung biopsy to confirm the diagnosis and eventually died from respiratory failure with a chest X-ray showing diffuse interstitial disease.

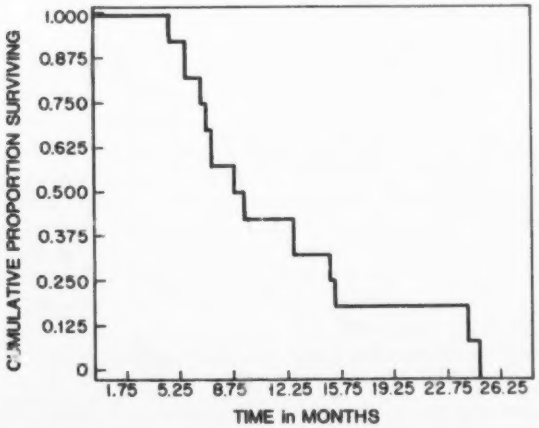

FIGURE 1.—Actuarial survival curve for all evaluable patients.

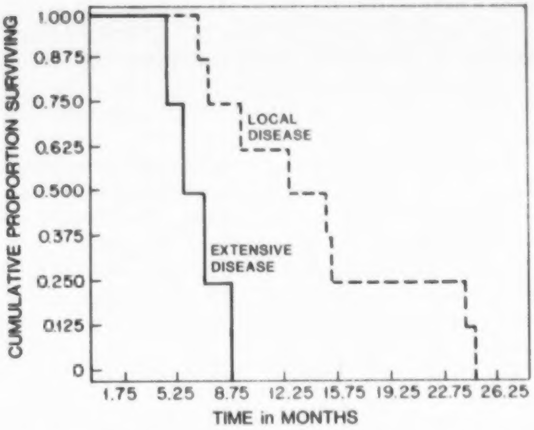

FIGURE 2.—Actuarial survival curves for extensive-disease patients compared to limited-disease patients.

Only two of 12 patients were able to receive maintenance chemotherapy without delay as well as in full dose. One of these patients died a respiratory death with bilateral radiation pneumonitis after three doses of maintenance chemotherapy; the other developed a brain metastasis after two cycles of chemotherapy and died 1 month later. Three patients never received maintenance chemotherapy because of chronic pancytopenia (one patient), severe unresolved malaise from induction therapy (one), and early progressive disease (one). The remaining seven patients either had delays in initiating maintenance followed by full doses (one patient) or attenuated doses (five patients) or had no delay in initiation of maintenance followed by dose reductions (one patient). Dose reductions ranged between 50% and 75% for either leukopenia or thrombocytopenia or both. Delays ranged between 1 and 2 weeks.

Seven patients relapsed first within the local radiation field, six relapsed at distant sites (two relapsed simultaneously in the chest and distantly), and one died a respiratory death before relapse. In three of the seven local recurrences, inadequate coverage by the local radiation fields may have accounted for the local failures. The median time to first relapse was 7 months (range, 3–14.5) and median survival of all patients was 9 months (range, 5–25) (fig 1). For the eight patients with initially limited disease, the median time to first relapse was 8 months and the median survival was 12 months (fig 2).

DISCUSSION

Half-body irradiation has been suggested as an innovative form of consolidation therapy in the treatment of SCBC (1,2,7,9). This form of "systemic irradiation" offers a 1–3-log cell kill that may substitute for a cycle of systemic chemotherapy (1). Concerns about its use include (*a*) the enhanced toxicity of combined aggressive chemotherapy and irradiation (7), (*b*) the danger of radiation pneumonitis, both acute and chronic (10), and (*c*) the risk of treatment delays that might undermine the control of systemic disease.

This pilot study reinforces some of the above concerns. As participants in the first pilot study utilizing lower doses of chemotherapy (7), we were already concerned about treatment delays and attenuation of maintenance therapy. However, in that study, toxicity was acceptable. In this study, combining higher doses of induction chemotherapy with UHBI and LCI resulted in unacceptable toxicity.

The toxicity of the radiation therapy program that immediately followed aggressive chemotherapy was high. Five patients required early rehospitalization, including one who developed chronic marrow hypoplasia and two who were quite ill and required prolonged hospital stays. Three patients with underlying chronic obstructive pulmonary disease developed radiation pneumonitis, one of whom died of diffuse interstitial pneumonitis and respiratory failure induced by the UHBI.

Delays in maintenance chemotherapy and dose reductions were necessary in most patients. No patient in this study received full-dose maintenance chemotherapy on schedule for $>$ 6 weeks.

Finally, and most disappointingly, despite the good PS of this group of patients, the high proportion of patients with limited disease (67%), and their initial excellent response rate (100% CR + PR for evaluable patients, with 50% CR), the survival results were poor. Seven patients relapsed locally within the first year, and the median time to relapse and median survival times were relatively short. Even analyzing the best subgroup (those with limited disease) did not show a significant benefit to this therapy. Distant failures occurred early in many patients within the UHBI portal: brain, liver, adrenals, and pleura.

Though more extensive local radiation portals and higher mediastinal X-ray doses may have decreased the high incidence of local failure, it would not have improved the pattern of complications seen and may have increased them. Thus, because of toxic effects, treatment delays, and poor survival in this predominantly favorable group of patients with SCBC, we cannot recommend this combined modality approach in patients with SCBC.

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Phase I-II Study of the Combination of 5-FU, Doxorubicin, Mitomycin, and Semustine (FAMMe) in the Treatment of Adenocarcinoma of the Stomach, Gastroesophageal Junction, and Pancreas[1]

David A. Karlin,* John R. Stroehlein, Roland W. Bennetts, Ronald D. Jones, Lawrence J. Heifetz, and Pradeep S. Mahal[2]

One hundred and fifteen patients with advanced gastrointestinal cancer (stomach cancer, 42 patients; gastroesophageal junction cancer, ten; pancreatic cancer, 32; and other upper gastrointestinal cancers, 31) were treated with a combination chemotherapy regimen consisting of 5-FU, doxorubicin, mitomycin, and semustine (methyl-CCNU) (FAMMe). Of the 31 patients with stomach cancer who were evaluable for response and had had no previous chemotherapy, 12 (39%) achieved complete or partial remission. One of eight (12%) patients with gastroesophageal junction cancer and five of 23 (22%) patients with pancreatic cancer achieved a partial remission. The median duration of survival for all patients with adenocarcinoma of the stomach was 7.1 months. The median duration of survival for responding patients with stomach cancer was 13.6 months, and the median survival for nonresponding patients was 6.1 months. FAMMe chemotherapy was generally well-tolerated and can be administered in adequate doses without producing prohibitive myelosuppression. The starting dose should be reduced for patients ⩾ 70 years old or for patients who have received pelvic or vertebral radiation therapy. FAMMe is effective against advanced gastric cancer; however, because this was not a randomized comparative study of the relative effectiveness of FAMMe and FAM (5-FU, doxorubicin, and mitomycin), no recommendation for the use of one regimen instead of the other for advanced adenocarcinoma of the stomach can be made. FAMMe chemotherapy cannot be recommended for advanced adenocarcinoma of the pancreas and gastroesophageal junction.

[Cancer Treat Rep 66:1613–1617, 1982]

Patients with adenocarcinoma of the stomach, gastroesophageal junction, or pancreas have a dismal prognosis. For all patients, the 5-year survival rate ranges from 10% for stomach cancer to < 1% for pancreatic cancer (1–3). For patients who undergo "curative" resection, the 5-year survival rate ranges from 20% for stomach cancer to < 5% for pancreatic cancer (1–3). Because most tumors are unresectable at the time of diagnosis and most resected cancers recur, effective chemotherapy regimens for the palliation of symptoms and prolongation of survival are needed.

Combination chemotherapy regimens using drugs active against gastric and pancreatic cancers have improved response rates when compared with those from single-agent chemotherapy (4–12). Combination chemotherapy offers improved survival for advanced gastric cancer (4) but not for pancreatic cancer (5,6,10,11). Most regimens are based on 5-FU plus one or more of the following drugs: a nitrosourea, doxorubicin, and mitomycin (4–12). The results of therapy with a four-drug regimen for advanced gastric cancer containing 5-FU, doxorubicin, mitomycin, and semustine (methyl-CCNU) (FAMMe), given over an 8-week cycle, were reported (13). The 11% objective response rate was worse than the response rate achieved with most other multidrug regimens, and the median survival was similar to the median survival reported for most other multidrug regimens (4–12). This disappointing finding might be the result of drug dose and scheduling.

Our study was designed to use a regimen with the same four drugs at different doses and a different schedule. The aims of our phase I-II study were to determine the maximum tolerable dose for the FAMMe combination chemotherapy regimen and to assess the efficacy of this regimen for treatment of advanced adenocarcinoma of the stomach, gastroesophageal junction, and pancreas.

PATIENTS AND METHODS

One hundred and fifteen patients with histologic confirmation of advanced upper gastrointestinal cancer were

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[1] Received Nov 2, 1981; revised Mar 4, 1982; accepted Apr 13, 1982.

[2] Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to*: David A. Karlin, MD, Department of Medicine, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

entered in the study between January 1979 and January 1981. Of these, 42 had adenocarcinoma of the stomach, ten had adenocarcinoma of the gastroesophageal junction, and 32 had adenocarcinoma of the pancreas. Adenocarcinoma of the gastroesophageal junction was considered to be a different disease than adenocarcinoma of the stomach (14,15). The remaining 31 patients had various other upper gastrointestinal cancers: eight patients, hepatocellular carcinoma; six, intestinal carcinoid; four, gallbladder; five, bile duct; two, ampulla of Vater; three, adenocarcinoma of the small intestine; two, pancreatic islet cell; and one, pancreatic acinar. The characteristics of the patients with adenocarcinoma of the stomach, gastroesophageal junction, or pancreas are shown in table 1. All patients gave written, informed consent prior to treatment, in accordance with institutional policy.

The dose and schedule for the FAMMe regimen are illustrated in table 2. During the phase I study, patients were entered at sequential starting doses. When the maximum tolerable dose was determined to be dose level C, most other patients were started at that level. Patients at high risk of hematologic toxicity, defined as being ≥ 70 years old, having had previous vertebral or pelvic radiation therapy, or having had prior therapy with alkylating agents, often started at dose level B. Dose modification for subsequent cycles was based on the wbc or neutrophil and platelet count nadirs during the preceding cycle. The dose of doxorubicin was reduced if liver function was impaired (table 1).

Because this was a phase I–II study, measurable lesions were not required; however, most patients entered were evaluable for response (table 1). Objective response was determined by physical examination, roentgenograms, radionuclide scans, computerized tomograms, ultrasonograms, and endoscopy. When radionuclide liver scans were used to quantitate response to chemotherapy, discrete areas seen on two views, single or multiple, > 2 cm in diameter with > 50% decrease in count rate were required. When endoscopy was used, indicator lesions were measured in reference to an open biopsy forceps. Measurement of response using these diagnostic modalities was performed by the consultants who performed the diagnostic study and also by one of the study investigators. A complete response was defined as no objective signs of residual cancer. A partial response was defined as a reduction of ≥ 50% in the product of the longest perpendicular diameters of the best measurable lesion; it was defined for liver metastasis as a reduction

TABLE 1.—Patient characteristics

	No. of patients		
Characteristic	Gastroesophageal junction adenocarcinoma	Stomach adenocarcinoma	Pancreatic adenocarcinoma
Entered	10	42	32
Nonevaluable	2	7	9
Evaluable for response	8	35	23
Mean age in yrs	55	57	59
Sex			
Male	7	21	13
Female	1	14	10
Metastasis			
Local	7	32	23
Liver	3	19	11
Lung	4	8	2
Abdomen	1	17	10
Bone	1	2	1
Nodes	2	4	3
Skin	1	2	1
Prior therapy			
Radiation therapy to tumor	3	2	1
Radiation therapy to pelvis or vertebrae	2	2	3
Chemotherapy	0	4	0
Performance status*			
0	1	13	5
1	3	11	6
2	1	10	4
3	3	1	8
4	0	0	0

*Eastern Cooperative Oncology Group criteria.

TABLE 2.—Schema for FAMMe regimen dose, schedule, and toxicity adjustments

a. Dose and schedule

	Dose (mg/m^2)				Day				
Drug, route	A	B	C	D	1*	8†	29*	36†	57(1)*
5-FU, iv	300	450	600	750	X	X	X	X	X
Doxorubicin, iv	15	22.5	30	37.5	X	—	X	—	X
Mitomycin, iv	5	7.5	10	10	X	—	—	—	X
MeCCNU, orally	50	75	100	125	X	—	—	—	X

b. Toxicity adjustments

All 4 drugs reduced for myelosuppression nadir				Doxorubicin only reduced for liver function		
Polymorphonuclear count (cells/mm^3)	or	Platelet count (cells/mm^3)	% dose reduction	Bilirubin (mg/100 ml)	or	SGOT (× normal)
750–1000	or	50,000–75,000	25	—		SGOT 2–3 ×
< 750	or	< 50,000	50	1.2–3		SGOT > 3 ×
—		—	75	> 3		—

*Minimum wbc count of 3500/mm^3 and platelet count of 100,000/mm^3 to start.

†Minimum wbc count of 2500/mm^3 and platelet count of 75,000/mm^3 to start.

of ⩾ 30% in the sum of the subcostal and subxyphoid measurements. In addition, a partial response required that there be no increase in any lesion and no new metastasis. Stable disease was defined as either no change or no regression or a < 25% increase in any lesion, and no new metastasis. Disease progression was defined as a ⩾ 25% increase in any lesion, or a new metastasis. To qualify as a response or stable disease the response or initial status had to be maintained for at least 8 weeks.

Survival and duration of response were determined from the start of therapy. Survival data were calculated by the Kaplan and Meier method (16) and statistically evaluated by the Gehan modification of the generalized Wilcoxon test (17). Chi-square statistical analysis was used to evaluate the possible relationship between the patient characteristics (table 1) and response to chemotherapy and survival. For four patients with adenocarcinoma of the stomach, Fisher's exact test for independence was used to evaluate the effect of the prior chemotherapy on subsequent response rates.

RESULTS

Objective Response

Twelve of 35 patients (34%) with adenocarcinoma of the stomach achieved a complete or partial response (table 3). The median duration of response was 6 months (range, 2–8). The median duration of survival for all patients with adenocarcinoma of the stomach was 7.1 months. The median duration of survival for responding and nonresponding patients was 13.6 and 6.1 months, respectively ($P < 0.006$).

Five of 23 patients (22%) with adenocarcinoma of the pancreas achieved a partial response (table 3). The median duration of response was 6 months (range, 2–8). The median duration of survival for all patients with adenocarcinoma of the pancreas was 7.4 months. The difference in the median survival time between responding and nonresponding patients was not statistically significant ($P = 0.48$).

One of eight patients (12%) with adenocarcinoma of the gastroesophageal junction achieved a partial remission (table 3) lasting 6 months. Twenty-one of the 31 patients with various other gastrointestinal cancers were evaluable for response. The partial response rates for these cancers were: $33^1/_3$% (two of six patients), hepatocellular carcinoma; 60% (three of five), intestinal carcinoid; 0 (none of three), gallbladder; 100% (one of one), bile duct; 0 (none of one), ampulla of Vater; 50% (one of two), adenocarcinoma of the small intestine; 50% (one of two), pancreatic islet; and 0 (none of one), pancreatic acinar.

Patient characteristics that potentially influenced response were analyzed (table 1). Sex, age, physical performance status, unresectable primary cancer, and sites of metastasis did not affect response rates for patients with adenocarcinomas of the stomach, gastroesophageal junction, and pancreas (χ^2). Radiation therapy to the unresected primary tumor had no effect on subsequent response rates for adenocarcinomas of the stomach, gastroesophageal junction, and pancreas (χ^2). None of the patients with adenocarcinoma of the gastroesophageal junction or pancreas received chemotherapy prior to entering this study. Four patients with adenocarcinoma of the stomach received prior chemotherapy. All four patients were nonresponders. For these four patients the effect on subsequent response rates was statistically significant (Fisher's exact test for independence). If these

TABLE 3.—Objective response

Response	No. of patients (%)		
	Gastroesophageal junction adenocarcinoma	Stomach adenocarcinoma	Pancreatic adenocarcinoma
Complete	0	1 (3)	0
Partial	1 (12)	11 (31)	5 (22)
Stable	2	11	11
Disease progression	5	12	7
Total	8	35	23

four nonresponders who had received prior chemotherapy are removed from the calculation of response rate, the complete plus partial response rate for stomach cancer is 39%.

Toxicity

The dose-limiting toxic effect was myelosuppression. Seventy-six percent of the 115 patients started at dose level C, 21% at level B, and 3% at level A. Subsequent dose adjustments were almost always downward, indicating the cumulative myelosuppression of this four-drug regimen. By the fourth chemotherapy cycle, 5% of the 19 remaining patients were treated with dose level D, 21% at level C, 32% at level B, and 42% at level A. Only one patient received nine cycles of chemotherapy.

There were two wbc count nadirs, between Days 20 and 26 and Days 38 and 43, and two platelet count nadirs, between Days 19 and 24 and Days 31 and 50. Severe myelosuppression was defined as a wbc count nadir $\leq$ 1000/mm^3, a neutrophil count nadir $\leq$ 500/mm^3, or a platelet count nadir $\leq$ 25,000/mm^3. Adequate data were available to evaluate 186 chemotherapy cycles for myelosuppression. Thirteen percent of these cycles were characterized by severe myelosuppression. There were two deaths related to severe myelosuppression. One of the patients had received radiation therapy to a significant volume of bone marrow prior to chemotherapy and both were $\geq$ 70 years old. Two pretreatment factors appeared to increase the risk of severe myelosuppression: age $\geq$ 70 years and radiation therapy.

DISCUSSION

The FAMMe regimen was generally well-tolerated. Although the dose-limiting toxic effect was myelosuppression, there were fewer dose modifications than with the four-drug regimen of Bunn et al (13). This was probably due to differences in drug dose and scheduling (18,19). Our regimen used more 5-FU and less doxorubicin, mitomycin, and semustine per 8 weeks. For cycles 1, 2, and 3 in the study of Bunn et al there were 28%, 67%, and 75% dose level modifications compared with 21%, 16%, and 20% for the FAMMe regimen.

The complete plus partial response rate for patients with adenocarcinoma of the stomach with no chemotherapy prior to this study was 39%. This is similar to the 42%–55% response rate to FAM (5-FU, doxorubicin, and mitomycin) reported by several groups (7,12,20). 5-FU, doxorubicin, and nitrosourea combinations have resulted in response rates of 52% (9). Regimens without doxorubicin for advanced gastric cancer employing 5-FU plus a nitrosourea or mitomycin produced response rates of 24%–40% (4,8).

For adenocarcinoma of the pancreas our partial response rate of 22% was disappointing. Response rates to FAM chemotherapy of 37%–40% have been reported (11,20). 5-FU, doxorubicin, and nitrosourea combinations have resulted in response rates of 26%–43% (6,10). Regimens without doxorubicin for advanced pancreatic cancer employing 5-FU plus a nitrosourea produce response rates of 12%–27% (5,21). It is unclear why we were unable to achieve a response rate similar to that from FAM chemotherapy for pancreatic cancer since none of our patients with pancreatic cancer had received prior chemotherapy, and the physical performance status and extent of disease were similar to the patients treated with FAM.

Sex, age, physical performance status, unresectable primary cancer, and sites of metastasis did not affect response rates for patients with adenocarcinoma of the stomach, gastroesophageal junction, and pancreas. This finding was somewhat unexpected since these patient characteristics often affect response to chemotherapy. They have been shown to affect response to 5-FU therapy in patients with advanced gastric cancer (4,22,23). In contrast to the single-drug studies, however, physical performance status and resectability of the primary cancer had no statistically significant effect on response and survival in patients with gastric cancer treated with FAM (7). The composition of our patient population may account for this unexpected finding. The effect of these patient characteristics on response and survival in patients treated with chemotherapy for adenocarcinoma of the pancreas is unclear because of insufficient published information.

We conclude that FAMMe chemotherapy can be administered in adequate doses without producing prohibitive myelosuppression. The starting dose was determined to be dose level C for most patients; for patients $\geq$ 70 years old or patients who had received pelvic or vertebral radiation therapy, the starting dose was level B (table 2). FAMMe chemotherapy is effective for advanced gastric cancer. Because this was not a randomized comparative study of the relative effectiveness of FAMMe and FAM chemotherapy, no recommendation for the use of one regimen instead of the other for advanced adenocarcinoma of the stomach can be made. FAMMe chemotherapy cannot be recommended for advanced adenocarcinoma of the pancreas and gastroesophageal junction.

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Phase I–II Evaluation of a New Anthracycline Antibiotic, Aclacinomycin A, in Adults With Refractory Leukemia[1,2,3,4]

Raymond P. Warrell, Jr,* Zalmen A. Arlin, Sanford J. Kempin, and Charles W. Young[5,6]

Aclacinomycin A (ACM) is a new anthracycline antibiotic that produces substantially less cardiotoxicity in animals than does doxorubicin. To define the effective dose for the treatment of patients with leukemia, we treated 43 patients with acute nonlymphoblastic leukemia (ANLL) or acute lymphoblastic leukemia (ALL) using ACM administered at three dose levels. All patients had previously received extensive treatment with other chemotherapy; their median cumulative dose of prior anthracycline was 340 mg/m². An ACM dose of 100 mg/m²/day given for 2 days (total dose, 200 mg/m²) failed to produce significant bone marrow hypocellularity or remission in two patients. Total ACM doses of 300–360 mg/m² (100 or 120 mg/m²/day x 3 days) produced marrow hypoplasia in 16 of 23 evaluable patients with ANLL. Overall, four of 32 patients with ANLL who received 300–360 mg/m² of ACM achieved complete remission for durations of 1, 5+, 6, and 15+ months. Two of nine patients with ALL achieved partial remission. Toxic effects of this therapy included severe leukopenia and thrombocytopenia, nausea, mucositis, and diarrhea. ECG abnormalities were noted in 43% of patients who were carefully monitored; however, only one patient developed a significant decrease in left ventricular ejection fraction as measured by radionuclide cardiography. ACM produced only minimal alopecia and did not cause tissue necrosis following inadvertent subcutaneous infiltration. We conclude that 300–360 mg/m² of ACM is an effective dose for the treatment of patients with ANLL and that further evaluation of this compound is indicated in patients who have received minimal prior therapy. [Cancer Treat Rep 66:1619–1623, 1982]

Anthracycline antibiotics used in combination with other agents can produce complete remission (CR) rates > 70% in previously untreated patients with acute nonlymphoblastic leukemia (ANLL) (1,2). However, the long-term administration of these compounds is limited by the development of cardiomyopathy, which is directly related to the cumulative dose (3). Since the anthracyclines have broad clinical utility, a major research effort has been directed toward the identification of active structural analogs that do not share the cardiotoxic effect.

Aclacinomycin A (ACM) is a new antibiotic initially isolated from strains of *Streptomyces galilaeus* by Oki et al (4). The chemical structures for ACM and the prototype anthracycline doxorubicin are presented in figure 1. Both compounds share similar planar anthraquinone rings but differ in that ACM contains three deoxy sugars as well as different substitutions at the 4, 10, 12, 14, and 5′ positions.

Preclinical evaluation of ACM has shown several important differences relative to doxorubicin. Cellular uptake of ACM occurs more rapidly than doxorubicin and final intracellular concentrations are higher (5). ACM is also a more potent inhibitor of RNA synthesis and is less mutagenic (6). In equal dosage, ACM is somewhat less effective against L1210 leukemia, Lewis lung carcinoma, and B16 melanoma; however, several in vivo studies have suggested that the cardiotoxic effect of ACM is as low as one tenth that of doxorubicin (7,8). Therefore, ACM might have a greater therapeutic index than doxorubicin.

Initial clinical trials of ACM in Japan revealed a pattern of toxic effects similar to that for doxorubicin; however, alopecia was minimal and congestive cardiomyopathy was

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[1] Received Feb 8, 1982; revised Apr 27, 1982; accepted May 10, 1982.

[2] Supported in part by Public Health Service grant CA-05826 and by contract N01-CM-57043 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

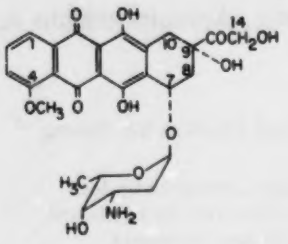
[3] Presented in part at the annual meeting of the American Association for Cancer Research, Washington, DC, April 26, 1981.

[4] Aclacinomycin A was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

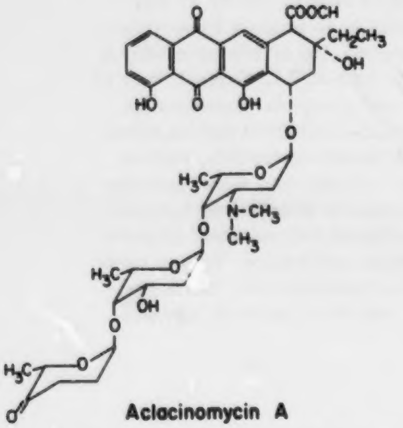
[5] Developmental Chemotherapy (*R. P. Warrell, Jr*, and *C. W. Young*) and Hematology/Lymphoma (*Z. A. Arlin* and *S. J. Kempin*) Services, Memorial Sloan-Kettering Cancer Center, and the Cornell University Medical College, New York, NY.

[6] We thank Ms. Catherine Cassidy for assistance with data collection, the Chemotherapy Research Nursing staff, and Ms. Shelley Hantman for preparing the manuscript.

**Reprint requests to:* Dr. Raymond P. Warrell, Jr, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.



Doxorubicin



Aclacinomycin A

FIGURE 1.—Structural formulae of doxorubicin and aclacinomycin A.

not observed (9,10). Antitumor effects were noted in patients with malignant lymphoma and carcinomas of the breast, ovary, and bladder. Following these studies, several groups initiated clinical studies in patients with leukemia. We report here the results of a phase I–II evaluation of ACM in patients with refractory acute leukemia.

PATIENTS AND METHODS

Patients

Adult patients with acute leukemia were eligible for entry in this study after they had failed standard chemotherapy for remission induction or had relapsed from a combination chemotherapy program used for remission maintenance. All patients had adequate hepatic and renal function (serum bilirubin and creatinine $\leq$ 1.5 mg/dl). There were no restrictions on the amount of prior chemotherapy (including treatment with anthracyclines) or on initial performance status (PS). The pretreatment evaluation included a bone marrow aspirate, cbc, screening biochemical profile, ECG, and chest roentgenogram. The initial evaluation also included a gated radionuclide cardiac scan (11); however, because of these patients' low PS, it was not feasible to perform stress radionuclide angiography. Accordingly, the initial study was a resting scan which was used only to determine eligibility for protocol entry. A resting left ventricular ejection fraction (LVEF) of $\geq$ 50% was established as one of the entry criteria. Signed, informed consent was required and the study was approved in advance by the center's Clinical Investigations Committee.

Patients were monitored with the following laboratory tests: cbc and platelet count daily; serum electrolytes, BUN, and glucose every other day; and screening biochemical profile every 7 days. While in the study, most patients had daily ECGs for the first 4–5 days of treatment, and where clinically feasible, patients underwent a repeat cardiac scan following recovery of their blood cell counts. Patients achieving CR were evaluated with stress radionuclide cardiography at intervals corresponding to 300 mg/m^2 of ACM (cumulative dose).

Assessment of Response

Response to ACM was evaluated at two points during this study. First, the ability of ACM to produce bone marrow hypoplasia was evaluated by a bone marrow aspirate and/or biopsy 7–10 days after the initiation of therapy. Marrow hypoplasia was defined as hypocellularity with no evidence of leukemic blast cells. Second, patients were evaluated for their ultimate clinical response. Patients in CR were required to have a cellular bone marrow with $<$ 5% blast cells, hemoglobin $>$ 10 g/dl, peripheral wbc count $\geq$ 3500/mm^3, and platelet count $\geq$ 100,000/mm^3. A partial response was defined as $\leq$ 10% bone marrow blast cells with the same peripheral blood cell criteria.

Treatment Protocol

Patients were entered in this study at three dose levels. The initial level represented twice the dose established by Casper et al (12) in a phase I study for the treatment of patients with solid tumors. Hospitalization was required for all patients receiving the induction chemotherapy. The prescribed dose of ACM was dissolved in 50 ml of a 5% dextrose solution and was administered as an iv infusion over 30–60 minutes.

RESULTS

Thirty-four patients with ANLL and nine patients with acute lymphoblastic leukemia (ALL) were entered in this study. The characteristics of the patients with ANLL are presented in table 1. All patients had received extensive prior chemotherapy for leukemia. All but two patients had had prior exposure to other anthracycline antibiotics (principally daunorubicin); the median cumulative dose of prior anthracycline was 340 mg/m^2. Less than one half of the patients had an outpatient PS at the time of entry (ie, Karnofsky PS $\geq$ 50). Fourteen patients (41%) were

TABLE 1.—Characteristics of patients with ANLL treated with ACM

Characteristic	
Median age, yrs (range)	41 (18–75)
No. of patients with Karnofsky PS of:	
50–60	16
30–40	18
Median No. of previous induction attempts (range)	2 (1–6)
Median No. of relapses (range)*	1 (1–4)
Median cumulative dose of prior anthracycline, mg/m² (range)	340 (0–580)

*Data exclude 5 patients who had never previously achieved remission.

receiving three or more antibiotics at the time of entry and 11 (32%) had immediately failed an induction attempt with combination chemotherapy. Twenty patients (59%) were treated with ACM at their first relapse; five had never achieved remission despite at least two attempts with other intensive combination chemotherapy programs (1,13). One patient was treated after relapsing from bone marrow transplantation.

Three dose levels were examined during this study (table 2). At a dose of 100 mg/m²/day x 2 days (200 mg/m²), two patients developed marked leukopenia (ie, wbc count $< 500/\text{mm}^3$), but neither achieved a significant degree of marrow hypocellularity or clinical remission. Sixteen patients then received 100 mg/m²/day x 3 days (300 mg/m²). At this level, ten of 15 evaluable patients achieved marrow hypoplasia and three achieved CR. In an effort to increase the percentage of patients achieving marrow hypoplasia, a final dose level of 120 mg/m²/day x 3 days was initiated. Six of eight evaluable patients at this level developed hypoplasia and one patient has had a CR.

Allowing no exclusions, four of 32 patients with ANLL who received ≥ 300 mg/m² of ACM achieved CR, for an overall CR rate of 13%. Despite the extensive prior therapy with other anthracyclines, 16 of 23 evaluable patients (70%) developed marrow hypoplasia at the two highest dose levels. Seven patients died within the first week of therapy before the marrow effects of ACM could be assessed; none of these deaths were believed to be drug-related. (Three patients died from hemorrhage, three died of sepsis, and one died from aspiration pneumonia.) Two other patients (both at the 300-mg/m² level) achieved a remission bone marrow but their platelet count failed to increase to $\geq 100{,}000/\text{mm}^3$. One of these patients died on Day 30 from an intracranial hemorrhage; the other died on Day 88 of fungal endocarditis. Neither patient had evidence of leukemia at the time of death. The median time needed to achieve marrow remission was 24 days (range, 19–30). We have only limited information regarding the activity of ACM in patients with ALL; to date, two of nine heavily pretreated patients with generally poor PS have achieved partial responses at a dose of 300 mg/m².

Patients achieving CR underwent "consolidation" therapy with ACM, receiving their induction dose again 6–8 weeks later. Patients in remission were maintained on ACM as a single agent, usually receiving 100 mg/m² every 3 weeks as outpatients. Two patients have received cumulative doses of ACM totaling 1100 and 2100 mg/m², having previously received 360 and 180 mg/m² of daunorubicin, respectively.

Toxicity

As expected, myelosuppression was severe. All patients who received ≥ 300 mg/m² (total dose) developed profound neutropenia and thrombocytopenia, and all patients required platelet transfusions and parenteral antibiotics.

Thirty-seven patients with either ANLL or ALL who received ≥ 300 mg/m² were closely monitored with daily ECGs during their first 4–5 days of treatment. As noted in table 3, 16 of those individuals (43%) developed transient ECG abnormalities consisting of T-wave inversion or ST-T segment depression. These abnormalities were not associated with symptoms and generally reversed within the first week of therapy. One 20-year-old patient developed dyspnea, interstitial pulmonary infiltrates, and fever after the third dose of ACM. These symptoms reversed following the administration of diuretics and antibiotics. Although not confirmed, it was believed that this episode was related to transient left ventricular failure induced by ACM. A followup cardiac scan was obtained on this individual but no interval decrease in his

TABLE 2.—Response of patients with ANLL to ACM

Dose level (mg/m²)	No. of patients	No. of early deaths*	No. aplastic/No. evaluable	No. of CRs	Duration of CRs (mos)
100 × 2	2	0	0/2	0	
100 × 3	16	1	10/15	3	1, 6, 15+
120 × 3	16	6	6/8	1	5+

*Occurring < 7 days from start of therapy.

TABLE 3.—Toxic effects of ACM in adults with acute leukemia

Toxic effect	No. of patients (%)
ECG abnormality ($n = 37$)	16 (43)
T-wave inversion	11
ST-T depression	5
Arrhythmia ($n = 43$)	1 (2)
Mucositis ($n = 43$)	23 (53)
1+	18
2+	5
Diarrhea ($n = 43$)	11 (26)
Median peak serum bilirubin concentration, mg/dl (range): 1.5 (0.3–9.6)	

LVEF was observed. One patient with a history of hypertension developed supraventricular tachycardia 6 days after receiving his first dose of ACM; however, the relation of this effect to drug administration is doubtful.

A total of 12 patients were re-evaluated by radionuclide cardiography following the induction attempt with ACM. One patient who received 200 mg/m^2 showed a decrease in his LVEF from 58% to 42%, which was not associated with symptoms of congestive heart failure. None of the other 11 patients (all of whom received ACM doses ≥ 300 mg/m^2) showed any significant decrease (ie, $\geq 10\%$) in LVEF after this therapy. Neither of the two patients receiving maintenance therapy with ACM to total cumulative doses of 1100 and 2100 mg/m^2 have shown any decrease in LVEF, despite their prior therapy with daunorubicin.

Other prominent toxic effects associated with the use of high ACM doses were mucositis and diarrhea (table 3). Nausea and/or vomiting were universal on the days of therapy, but the nausea was not considered more severe than is commonly seen with other anthracyclines. Because many patients entered this trial with hepatocellular dysfunction related to prior therapy or transfusions, hepatoxicity was somewhat difficult to assess. Fourteen patients developed reversible increases in serum bilirubin concentration to levels > 2.0 mg/dl; this increase was usually associated with mild elevations in serum alkaline phosphatase activity. Alopecia was minimal and most patients had regrowth of hair during the treatment period. There were at least two occurrences of inadvertent subcutaneous drug infiltration; however, neither patient developed skin ulceration or necrosis.

DISCUSSION

We found ACM to be an effective drug for achieving bone marrow hypoplasia and CR in patients with ANLL. The CR rate of 13% is comparable to rates achieved by other investigators using doxorubicin in heavily pretreated patients, most of whom had never received other anthracyclines (14,15). Moreover, this rate is comparable to that which we recently reported for the use of AMSA in a similar patient population (16). Only one of the 43 patients entered in this trial subsequently achieved a CR with other chemotherapy, indicating the advanced and resistant state of their disease.

From our study, it appears that cumulative ACM doses of 300–360 mg/m^2 are necessary for patients to achieve CR. This closely agrees with results found by French and Japanese investigators (17,18). Both of these studies involved lower daily doses of ACM given for more prolonged periods (ie, 10–30 mg/m^2 for 10–14 days) and have shown CR rates ranging from 17% to 23% in patients who were previously treated with other chemotherapy.

The incidence of ECG abnormalities in this study is higher than that recorded by DeJager et al (18). It is unclear whether this incidence is related to closer ECG monitoring or to the differences in dose scheduling noted above. A high incidence of ECG abnormalities was recently reported following the administration of doxorubicin to individuals who were then continuously monitored for 24 hours (19). However, there is no evidence that these changes are correlated with the subsequent development of cardiomyopathy (3).

Despite suggestions to the contrary (20), we have found no indication in this study or a related one in patients with malignant lymphoma (21) that ACM lacks cross-resistance to other anthracyclines. This finding agrees with in vitro studies (22).

ACM was brought to clinical trial with the hope that it could equal or exceed the current therapeutic results of other anthracyclines with reduced toxicity. A randomized trial comparing ACM to other anthracyclines will be required to finally decide these issues. However, results from three studies now suggest that ACM is probably as effective as other analogs in the treatment of relapsed leukemia (17,18). These studies have also shown a very low incidence of cardiotoxicity as well as a lack of alopecia and tissue necrosis after subcutaneous extravasation. Further evaluation of this agent, both alone and in combination, is clearly warranted in minimally pretreated patients with acute leukemia.

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Vomiting Induced by Cyclophosphamide and Phosphoramide Mustard in Cats[1,2,3]

John H. Fetting,[4,5,*] Lawrence E. McCarthy,[6] Herbert L. Borison,[6] and Michael Colvin[4]

Cyclophosphamide and phosphoramide mustard produce significant vomiting. Cyclophosphamide is metabolized to phosphoramide mustard, which may ultimately contribute to vomiting after cyclophosphamide administration. The role of the chemoreceptor trigger zone (CTZ) in vomiting caused by these agents is unknown. We studied the emetic syndromes produced by iv and intracerebroventricular cyclophosphamide and phosphoramide mustard in unanesthetized normal and CTZ-ablated cats. Iv cyclophosphamide produced vomiting unpredictably, with a mean latency of 54 ± 9 mins (mean ± SE) in cats that vomited. A dose-response relationship was found for phosphoramide mustard-induced emesis. A dose of 200 mg/kg was consistently effective, with a mean latency of 127 ± 6 mins. Neither agent produced predictable emesis by the intracerebroventricular route of administration. One of four CTZ-ablated cats vomited after 300 mg/kg of cyclophosphamide. Since cyclophosphamide was an unpredictable emetic stimulus, it was not possible to further evaluate the effect of CTZ ablation on cyclophosphamide-induced vomiting. However, CTZ-ablated cats given 200 mg/kg of phosphoramide mustard vomited significantly less frequently ($P = 0.05$ by chi-square test) and with a longer latency than nonablated animals. A temporary, severe neurotoxic reaction was observed in cats receiving ≥ 300 mg/kg of cyclophosphamide, which may have had an inhibitory effect on emesis. Phosphoramide mustard was found to be a potent emetic stimulus in cats and may contribute to the emetic response following cyclophosphamide administration. Analysis of latency data suggests that in the cat other cyclophosphamide metabolites may also contribute to the emetic syndrome. [Cancer Treat Rep 66:1625–1629, 1982]

Nausea and vomiting occur very frequently after high-dose cyclophosphamide treatment (1–3). The mechanism of this emetic syndrome is unknown. A recent study of nausea and vomiting in 25 patients who received 50–75 mg/kg of cyclophosphamide by iv infusion demonstrated a 6–12-hour delay in symptom onset after drug administration (4). The prolonged vomiting latency after cyclophosphamide administration may be related to its metabolism. Cyclophosphamide itself demonstrates negligible cytotoxicity but is converted in the liver to cytotoxic metabolites (5). It has been suggested that the metabolite, phosphoramide mustard, produces emesis after cyclophosphamide treatment (5). The prolonged latency could be related to the time required for production of phosphoramide mustard, which was a potent emetic stimulus in clinical trials (6).

Vomiting resulting from a variety of drugs, including some cytotoxic agents, is mediated by the medullary chemoreceptor trigger zone (CTZ) (7,8). Vomiting may also be mediated by central and peripheral afferents to the vomiting center bypassing the CTZ (7). The role of the CTZ in vomiting caused by cyclophosphamide and phosphoramide mustard has not been established. In studies on the related compound mechlorethamine, however, Borison et al (9) were able by CTZ ablation to prevent emesis in the dog but not in the cat (9). Thoracic spinal cord transection, vagotomy, and abdominal sympathectomy were sufficient to prevent emesis in the cat.

We report here our studies on emesis after iv and intracerebroventricular (icv) administration of cyclophosphamide and phosphoramide mustard in cats. We show that phosphoramide mustard is a more potent emetic stimulus than cyclophosphamide in the cat. We suggest that vomiting caused by cyclophosphamide in the cat may not be solely related to circulating phosphoramide mustard. CTZ abla-

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[1] Received Sept 14, 1981; revised Mar 25, 1982; accepted May 13, 1982.

[2] Supported in part by Public Health Service grants CA-17970 and CA-25190 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by American Cancer Society Institutional Research grant IM 11Q. Dr. Fetting was the recipient of an American Cancer Society Regular Clinical Fellowship during the year 1980–1981.

[3] This work was carried out while Dr. Fetting was a Research Associate in the Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.

[4] The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD.

[5] Department of Psychiatry, The Johns Hopkins Hospital, Baltimore, MD.

[6] Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.

[*] *Reprint requests to:* Dr. John Fetting, The Johns Hopkins Oncology Center, 600 N Wolfe St, Baltimore, MD 21205.

tion significantly changed the course of emesis caused by phosphoramide mustard. Since cyclophosphamide was not a good emetic stimulus in the cat, we could not make a definite judgment on the role of the CTZ in emesis produced by this agent.

METHODS

Forty healthy adult cats were used for these studies. Surgical procedures were performed with sterile technique using xylazine and pentobarbital anesthesia. A combination of penicillin and streptomycin was routinely injected im postoperatively. Animals were fully recovered from surgery before use. Unless otherwise noted, animals received one drug dose and were killed at the end of the observation period.

We attempted to determine the dose-response relationship between iv cyclophosphamide and phosphoramide mustard and emesis. Initial doses were based on the reported frequency of vomiting after administration of cyclophosphamide and phosphoramide mustard in man (4,6). Depending on the vomiting response to the initial dose, drug doses were incrementally increased or decreased to determine the dose-response relationship. For iv evaluations of cyclophosphamide, a jugular venous catheter was inserted in each cat at least 48 hours prior to cyclophosphamide infusion through the indwelling catheter. Phosphoramide mustard was administered by bolus iv injection. We gave cyclophosphamide by indwelling catheter infusion because of the larger volume involved and to minimize the acute toxicity noted with more rapid administration of cyclophosphamide (*see* below).

The most predictably emetic dose of each agent was then used for tests on CTZ-ablated cats to determine the effect of this lesion on the course of emesis. CTZ ablation was performed with gentle thermal cauterization as described in a previous publication (10). Failure to vomit after 160 μg/kg of lanatoside C iv confirmed the success of CTZ ablation (11). CTZ-ablated animals were Formalin-perfused for histologic confirmation of the lesions (12).

Icv injections were made in an attempt to produce emesis predictably by another route of administration. For icv injections, cats were provided with an intracranial cannula placed stereotaxically in the right lateral ventricle (13). Vomiting induced by the injection of apomorphine (0.25 mg in 0.25 ml) served as a functional confirmation of proper cerebroventricular cannulation. Icv injections were delivered in 0.25–0.75 ml. The drug dose was incrementally elevated until overt drug effects were seen. Animals were then observed for 24 hours. Cannulae were flushed with saline at the end of the observation period. These cats fully recovered from an icv drug administration and were subsequently given higher doses.

An emetic response was scored as an all-or-none event characterized by the expulsion of gastric contents. Response latency was measured as the time between initiation of drug injection and the first emetic event. Initially, animals that received iv cyclophosphamide were observed overnight for emesis. When it became clear that late emesis did not occur if an animal had not vomited within 3 hours, a cutoff observation period of 3 hours was established. If emesis occurred within 3 hours, cats were observed for several hours thereafter to further characterize the emetic course. Cats that received iv phosphoramide mustard were observed for a minimum of 6 hours. As noted above, animals that received icv injections were observed for 24 hours.

Cyclophosphamide was contributed by Mead Johnson Incorporated and phosphoramide mustard by James C. Craddock of the National Cancer Institute. Drugs were dissolved in saline just prior to administration.

RESULTS

Iv Drug Administration

Table 1 shows the relationship between increasing doses of cyclophosphamide and phosphoramide mustard and vomiting. Cyclophosphamide failed to produce emesis reliably at any dose level but phosphoramide mustard showed a dose-response relationship.

Since cyclophosphamide did not produce consistent vomiting we have combined the entire sample of cats for further analysis of the vomiting latency. Cyclophosphamide over a dose range of 150–450 mg/kg iv produced vomiting in four of 17 cats, with a latency of 54 ± 9 mins (mean ± SE). Cats vomited one to three times over a maximal period of 4 hours from the start of the infusion. By comparison, a 200 mg/kg dose of phosphoramide mustard iv produced predictable vomiting in four of four animals, with a mean latency of 127 ± 6 mins. Cats vomited three to six times over the 6-hour observation period.

Licking movements and salivation were noted 5 mins after initiating administration of both drugs at all doses. In one animal given 750 mg/kg of cyclophosphamide and in three given 200 mg/kg of phosphoramide mustard, there was gagging and a single vomiting episode within 3–4 mins after the start of drug administration. Since repeated vomiting developed 2 hours after drug administration, we have chosen to enter the longer latency in the tables, and the prompt effects were considered to be spurious events.

Urinary frequency developed in all cats approximately 1 hour after initiation of cyclophosphamide administration. All three animals autopsied showed a severe hemorrhagic cystitis. Although urinary frequency was not noted with phosphoramide mustard, both animals autopsied after a dose of 200 mg/kg showed hemorrhagic cystitis.

An unexpected finding in all cats that received ≥ 300 mg/kg of cyclophosphamide was a profound neurotoxic effect. This was characterized by head bobbing, broad-based stance, and falling. It was first noted 10 mins after

TABLE 1.—Cyclophosphamide- and phosphoramide mustard-induced emesis in cats

Dose (mg/kg iv)	No. of cats tested	No. that vomited	Latency (mins)	Other
Cyclophosphamide				
150	4	1	45	Licking, salivation
300	6	2	54, 38	Licking, salivation, neurotoxicity
450	4	1	79	Licking, salivation, neurotoxicity
600–700	3	0	—	Licking, salivation, neurotoxicity, early emesis in 1 cat
Phosphoramide mustard				
50	2	0	—	Licking, salivation, hyperpnea
100	4	2	120, 360	Licking, salivation, hyperpnea
200	4	4	122, 126, 115, 144	Licking, salivation, early emesis in 3 cats, hyperpnea

the initiation of the infusion, increased in severity over the next 20–30 mins, and resolved over 1–2 hours. This was not seen with phosphoramide mustard but all animals did develop hyperpnea with drug administration, which resolved over 2–3 hours.

Icv Drug Administration

Neither cyclophosphamide nor phosphoramide mustard was an effective emetic stimulus by the icv route of administration but both had significant toxicity. A dose of 1.0–2.0 mg of cyclophosphamide failed to produce emesis in two cats tested. Licking and salivation were not evident. However, neurotoxic reactions characterized by nystagmus, broad-based stance, and falling was noted immediately after injection and resolved over 5–10 mins. The animals recovered fully. Phosphoramide mustard at doses of 0.025–5.0 mg produced emesis in only one of 11 trials. This was a single emetic episode after a dose of 0.75 mg. There was no vomiting at higher doses. Neurotoxic effects, licking, and salivation were produced in all cats at doses $\geqslant$ 0.75 mg. At doses $\geqslant$ 2.5 mg, cats developed anorexia, malaise, hyperpnea, extremity weakness, and spasticity. These doses were fatal in two cats at 35 hours, and animals were subsequently killed at 24 hours.

Since vomiting was not produced predictably by icv cyclophosphamide or phosphoramide mustard, CTZ-ablated animals were not tested using this route of administration.

Effect of CTZ Ablation on Vomiting

Table 2 shows the effect of CTZ ablation on emesis after administration of 300 mg/kg of cyclophosphamide or 200 mg/kg of phosphoramide mustard. One of four CTZ-ablated cats vomited after receiving cyclophosphamide, suggesting that CTZ ablation does not abolish emesis caused by this agent. However, since cyclophosphamide was a poor emetic stimulus and the number of cats that vomited was small, the effect of CTZ ablation on cyclophosphamide-induced emesis is not clear. Table 2 definitely shows that CTZ ablation did not totally prevent emesis after injection of phosphoramide mustard either. Nevertheless, the difference in the number of cats that vomited is statistically significant at the 0.05 level of probability by chi square.

The vomiting latency in the one CTZ-ablated animal that vomited in response to phosphoramide mustard was significantly longer than the emetic latency in intact animals. We observed no spurious early gagging or vomiting response to phosphoramide mustard in the CTZ-ablated animals. Two of three CTZ-ablated animals developed myoclonic jerks and dystonic posturing coincident with drug infusion. This resolved in 3 mins. One of these two animals was the only CTZ-ablated animal to vomit.

DISCUSSION

Emetic Activity

Iv cyclophosphamide produced vomiting in cats, with a mean latency of 54 ± 9 mins and a maximum duration of about 4 hours. The vomiting in man after large doses of iv cyclophosphamide has a latency of 6–12 hours and lasts 12–18 hours (4). In man the half-life of cyclophosphamide is about 7 hours (14). Although we do not know the half-life of cyclophosphamide in the cat, half-lives of 15–60 minutes have been reported for most animals examined (5). It has been proposed that the hemorrhagic cystitis seen after cyclophosphamide is caused by a metabolite (5). In the present study, the onset of cystitis symptoms about 1 hour after drug administration suggests that significant metabolism of

TABLE 2.—Effect of CTX ablation on emesis after iv cyclophosphamide and phosphoramide mustard in cats

Drug		No. of cats tested	No. that vomited	Latency (mins)
Cyclophosphamide (300 mg/kg)	Normal	6	2*	54, 38
	CTZ ablated	4	1*	119
Phosphoramide mustard (200 mg/kg)	Normal	4	4†	127 ± 6
	CTZ ablated	3	1†	181

*Not significant by chi-square test

†$P = 0.05$ by chi-square test.

cyclophosphamide had taken place by that time. This further supports the position that metabolism of cyclophosphamide is much more rapid in the cat than in man. Such accelerated metabolism may explain both the rapid onset and short duration of emesis in the cat.

Why cyclophosphamide did not produce dose-dependent emesis in the cat is unclear. It is possible that the observed neurotoxic effect is a manifestation of a more general toxicity that also inhibits emesis. Since the neurotoxic reactions lasted only 1–2 hours, one might reasonably expect vomiting to increase as neurotoxicity resolved. This might not occur, however, if the metabolism of cyclophosphamide were significantly more rapid in the cat than in man, as was proposed above. In that case, the emetic potential of cyclophosphamide and its metabolites might never be realized in the cat, since their levels would be highest at a time when an inhibitory neurotoxicity was most evident. It is also possible that dose-dependent emesis did not develop after cyclophosphamide administration because the cat does not produce adequate concentrations of emetic metabolites.

Phosphoramide mustard is a more potent emetic stimulus in the cat than is cyclophosphamide. Although the phosphoramide mustard metabolite generated from cyclophosphamide is theoretically able to cause emesis in the cat, it is not certain that the emesis following cyclophosphamide administration is necessarily caused exclusively by the phosphoramide mustard metabolite. Indeed, the vomiting latency after cyclophosphamide was 54 ± 9 mins, whereas the latency after phosphoramide mustard at 200 mg/kg was more than twice as long, ie, 127 ± 6 mins. The longer latency of emesis following phosphoramide mustard suggests that in the cat, cyclophosphamide itself or some metabolite other than phosphoramide mustard may play a role in emesis. It has been proposed that 4OH cyclophosphamide is transported into cells, where it decomposes to phosphoramide mustard (14). Vomiting following cyclophosphamide in the cat may ultimately be caused by phosphoramide mustard, but the latency may be related to the form in which it enters cells. In man, pharmacokinetic studies have shown that after cyclophosphamide administration, maximum levels of phosphoramide mustard are achieved in 2–3 hours and the plasma level of this metabolite remains elevated over the next 4–6 hours (15). Phosphoramide mustard has been shown to be a potent emetic stimulus in the present study and in clinical trials in man (6). For the cats in the present study and for man, a 2-hour latency was noted between drug administration and vomiting (6). Thus, vomiting after cyclophosphamide, which has delayed onset and is protracted, could be understood in terms of its metabolism to phosphoramide mustard. It is uncertain if other metabolites may contribute to vomiting after cyclophosphamide administration in man.

The gagging and vomiting episodes observed in a few animals within 3–4 mins of cyclophosphamide and phosphoramide mustard administration do not seem to have a clinical counterpart. Prompt emesis was noted in toxicologic studies of mechlorethamine and may have been mediated by a cholinergic mechanism (16), which may contribute to the acute lethality in mice after cyclophosphamide (17). This very early emetic response may therefore be related to cholinergic effects of cyclophosphamide and phosphoramide mustard when given in very large doses and therefore may have no clinical counterpart.

Neurotoxicity

The head bobbing, broad-based stance, falling, and nystagmus seen after iv and icv cyclophosphamide in the cat have not been previously described. They may be caused by the parent compound since they are first noted 10 mins after the start of the infusion and after icv injection, where cyclophosphamide metabolism would be minimal. A similar syndrome has been reported following administration of mechlorethamine but the mechanism has not been elucidated (16). Alterations in the EEG have been demonstrated during infusions of very large doses of cyclophosphamide (18). Patients have reported neuropsychiatric symptoms during rapid cyclophosphamide infusions. (19). Myoclonic jerks and dystonic posturing were observed in two CTZ-ablated cats at the completion of bolus iv injections of 200 mg/kg of phosphoramide mustard. These symptoms resolved in 3 mins. Neurotoxicity was not reported with this compound in human clinical trials (6) or in toxicologic studies of mechlorethamine (16). The neurotoxicity produced by icv phosphoramide mustard was delayed in onset and was unremitting. A similar syndrome was observed with doxorubicin in the monkey and may have been related to a necrotizing angiopathy observed pathologically (20).

Role of the CTZ

We were unable to fully assess the role of the CTZ in vomiting after cyclophosphamide administration since we did not find a reliable emetic dose. However, since one of four CTZ-ablated animals vomited, the CTZ appears not to be essential for emesis after cyclophosphamide. Vomiting after phosphoramide mustard was less frequent and occurred with a prolonged latency after CTZ ablation. The vomiting observed may have been mediated by a non-CTZ mechanism. CTZ ablation failed to prevent emesis following mechlorethamine in the cat, although it did prolong the latency in a number of animals, thereby suggesting an ancillary role for the CTZ (9). On the other hand, mechlorethamine emesis in the cat was abolished by gut denervation and cord transection. This indicated that mechlorethamine acts peripherally and produces vomiting via visceral afferents. Vomiting following total-body irradiation is mediated by both CTZ and non-CTZ mechanisms (21,22). Phosphoramide mustard emesis also may be produced by more than one mechanism and, like mechlorethamine, be "radiomimetic." CTZ-mediated emesis produced by agents other than anticancer drugs typically have latencies < 30 mins (11,23–25). This is in contrast to the 120-min latency observed with phosphoramide mustard or 70-min latency reported with the CTZ-mediated emesis caused by cisplatin (8). It is possible that phosphoramide mustard and cisplatin have a slow cumulative action at the CTZ. Alternatively, these drugs may act elsewhere, producing an agent that then acts at the CTZ.

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Combination Chemotherapy With Cyclophosphamide and Ftorafur Against Advanced L1210 Murine Leukemia[1,2]

V. Bruce Grossie, Jr,* Michael G. Rosenblum, and Ti Li Loo[3]

The antitumor activity of cyclophosphamide (CP) in combination with ftorafur (FT) against advanced L1210 murine leukemia was investigated and compared with the activity of the combination of CP and 5-FU. CP at doses of 200–350 mg/kg was highly active, the most effective dose (% treated/control = 327) being 300 mg/kg. The activity of FT up to the LD10 dose and of 5-FU at 50 mg/kg was minimal (% treated/control ≤ 142). Coadministration of 200 mg/kg of FT with 100 mg/kg of CP resulted in an increase in activity as compared with the respective dose of CP alone as did the coadministration of 400 mg/kg of FT with 200 mg/kg of CP; the activities, however, were no better than that resulting from treatment with 300 mg/kg of CP alone. The coadministration of CP and 600 mg/kg of FT resulted in an increase in host toxic reactions and loss of antitumor activity, as did the coadministration of 400 mg/kg of FT with 300 mg/kg of CP. Treatment with FT at 400 or 600 mg/kg 24 hours before or after administration of CP at 200 or 300 mg/kg did not increase the activity over that occurring when the respective combinations of the drugs were coadministered. The activity of the combination of CP with 5-FU at doses previously reported to be synergistic was equal to that observed when mice were treated with 300 mg/kg of CP alone. These data suggest that FT increases the cytotoxicity of CP but that the combination is not synergistic. [Cancer Treat Rep 66:1631–1634, 1982]

The antitumor activities of cyclophosphamide (CP) and 5-FU have been studied in considerable detail. In numerous clinical trials these agents have been shown to be effective alone and in combination with other agents against a wide variety of human tumors (1–3). The combination of 5-FU and CP has shown favorable preclinical results against several tumor models (4–8); it has therefore been suggested that this combination is potentially synergistic. Ftorafur (FT) is a structural analog of 5-FU, its activity being similar to 5-FU but less myelosuppressive. The toxicity of FT is similar to that of a slow infusion of 5-FU (9). Recent reports suggest that the cytotoxicity of FT may involve metabolites of FT other than 5-FU (10,11). FT has recently been reported to be active against colorectal and breast tumors resistant to 5-FU (11). We have therefore studied the antitumor activity of FT in combination with CP to determine whether FT offers increased therapeutic advantage over 5-FU alone or in combination with CP.

METHODS

Male BDF_1 mice from Simonsen Laboratories, Gilroy, CA, were used. The mice were housed in plastic cages with woodchip bedding and allowed at least a 7-day acclimation period prior to use. All mice were allowed food and water ad libitum from the time of receipt to the end of the experiments. FT was obtained from the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, and CP was obtained from Mead-Johnson and Co, Evansville, IN. Drugs were administered ip as a saline solution at 0.1 ml/10 g of body weight. Except where specified, all drugs were administered simultaneously via separate syringes.

Mice were inoculated ip with 10^6 L1210 murine leukemia cells from the laboratory stock, which was maintained by weekly transfer of 10^5 cells in DBA_2 mice. On Day 4 after tumor implantation, all mice were pooled in a common cage, weighed individually, and assigned to

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[1] Received Dec 18, 1981; revised May 5, 1982; accepted May 13, 1982.

[2] Supported in part by Public Health Service grant CA-11520 and by contract N01-CM-87185 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Departments of Developmental Therapeutics *(V. B. Grossie, Jr,* and *T. L. Loo)* and Clinical Immunology and Biological Therapy *(M. G. Rosenblum)*, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* V. B. Grossie, Jr, PhD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Antitumor activity of FT and CP against advanced ascites L1210 murine leukemia

Dose (mg/kg)			Survival	
FT	CP	No. of mice	Mean ± SE (days)	% T/C
200	0	7	8.9 ± 0.4*	124
400	0	7	9.6 ± 0.4*	134
600	0	6	10.0 ± 0.6*	140
0	100	7	13.7 ± 1.2*	200
0	200	6	16.8 ± 0.4*,†	236
0	300	20	22.7 ± 1.6*,†	327
0	350	7	20.0 ± 4.5*	282

*Significantly different ($P < 0.05$) from value for respective saline-treated control.
†Significantly different ($P < 0.05$) from value for next lower dose of respective drug.

groups of six to eight mice with a weight range of ≤ 3 g. Treatment was initiated on Day 4, the average weight of each group being used to determine the volume of drug solution to administer per mouse. Each separate experiment included a saline-treated control, the survival of which ranged from 6.9 ± 0.3 to 7.3 ± 0.2 days. In the final experiment, the average body weight of mice on Day 4 was used to determine the volume of drug solution to administer on Days 4 and 5. The experiments were terminated when all mice had died or at 45 days after the last treatment. The % T/C was calculated from the mean survival of drug-treated mice (T) and saline-treated mice (C) for the respective experiment. The survival times (days) of the groups were compared using the Student t test.

RESULTS

CP was very active against the advanced ascites L1210 murine leukemia, each successive increase in the dose up to 300 mg/kg resulting in a significant increase in activity (table 1). The 300-mg/kg dose of CP was therefore considered the maximum effective dose for this study. FT resulted in a significant increase in survival over the control at 200 mg/kg, but further increases up to the LD10 dose (12) resulted in only a slight increase in survival.

The effect of combinations of FT and CP is shown in table 2. Coadministration of 200 mg/kg of FT with 100 mg/kg of CP and the combination of 400 mg/kg of FT with 200 mg/kg of CP resulted in a significant increase in survival over that for mice treated with the respective doses of CP alone. Coadministration of 600 mg/kg of FT with 100, 200, and 300 mg/kg of CP resulted in a significant decrease in survival as compared to the values for the respective doses of CP alone. This also occurred when 400 mg/kg of FT was coadministered with 300 mg/kg of CP. Increasing the CP dose from 100 to 300 mg/kg in combination with 100 mg/kg of FT resulted in a consistent increase in survival compared with the value for FT alone but was not significantly greater from that obtained by treatment with 300 mg/kg of CP alone.

The activity of FT is thought to be due to the slow release of 5-FU. The activity of 5-FU and CP at doses previously reported to be synergistic is therefore re-

TABLE 2.—Antitumor activity of FT coadministered with CP against advanced ascites L1210 murine leukemia

Dose (mg/kg)			Survival	
FT	CP	No. of mice	Mean ± SE (days)	% T/C
100	100	6	8.7 ± 1.3*	126
200	100	7	19.7 ± 0.7*,†,‡	287
400	100	6	12.8 ± 2.0†,‡	187
600	100	6	9.8 ± 1.3*,†	143
100	200	6	16.5 ± 3.3†,§	241
200	200	6	19.0 ± 2.4†	277
400	200	7	19.9 ± 0.8*,†	278
600	200	7	12.0 ± 2.2*,†,‡	168
100	300	6	25.5 ± 0.8†,§	372
200	300	14	22.6 ± 1.7†	323
400	300	7	11.9 ± 1.7*,†,‡,§	166
600	300	7	8.6 ± 0.8*	120

*Significantly different ($P < 0.05$) from value for respective dose of CP alone.
†Significantly different ($P < 0.05$) from value for respective saline-treated control.
‡Significantly different ($P < 0.05$) from value for next lower dose of FT within same dose of CP.
§Significantly different ($P < 0.05$) from value for next lower dose of CP within same dose of FT.

TABLE 3.—Antitumor activity of 5-FU alone or in combination with CP against advanced L1210 murine leukemia

Dose (mg/kg)			Survival	
5-FU	CP	No. of mice	Mean ± SE (days)	% T/C
50	0	7	9.7 ± 0.2*	142
50	100	7	20.9 ± 1.6*,†	304
50	200	7	19.1 ± 2.8*,†	274
0	300	14	22.6 ± 1.6*,†	327

*Significantly different ($P < 0.05$) from value for saline-treated controls.

†Significantly different ($P < 0.05$) from value for group treated with 50 mg/kg of 5-FU alone.

ported for comparison (table 3). The activity of the combination of 5-FU and CP was greater than that for 5-FU alone but was only about equal to that of 300 mg/kg of CP.

Mulder et al (7) reported that simultaneous treatment with 100 mg/kg of 5-FU and CP was the optimal sequence of administration against advanced L1210 murine leukemia in CDF_1 mice. Since FT is thought to be a slow-release form of 5-FU, allowing a 24-hour interval between administration of FT and CP might ameliorate the host toxicity and thereby increase the therapeutic efficacy of the combination. However, no significant benefit in survival was obtained by allowing a 24-hour interval between treatment with 400 or 600 mg/kg of FT and 200 or 300 mg/kg of CP (table 4).

DISCUSSION

The activity of FT or 5-FU against advanced L1210 leukemia was minimal although survival time was significantly increased over that of the saline-treated controls. The lesser activities of CP at 350 mg/kg, the combination of 600 mg/kg of FT with all doses of CP, and the combination of 400 mg/kg of FT with 300 mg/kg of CP were due to toxic reactions, the deaths occurring in mice that were in a depleted state and showed no signs of tumor. Although two of the combinations of FT and CP resulted in significantly increased survival over that for the respective doses of CP alone, the resulting activity was no greater than that when 300 mg/kg of CP was used alone. The activity of the combination of 5-FU at 50 mg/kg and CP at 200 mg/kg was equivalent to that previously reported (6). Although the optimal dose of CP was the same as reported by Fernandes and Klubes (6), the activity of the 300-mg/kg dose against advanced L1210 murine leukemia was greater in our studies.

These results suggest, therefore, that FT increases the cytotoxicity of CP but does not increase antitumor activity against advanced L1210 murine leukemia over that expected from treatment with the optimal dose of CP alone.

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TABLE 4.—Effect of sequence of FT and CP administration on antitumor activity against advanced L1210 murine leukemia

Dose (mg/kg)			Survival	
FT	CP	Sequence of administration*	Mean ± SE (days)†	% T/C
400	200	FT + CP	24.4 ± 1.0	335
400	200	FT → CP	17.4 ± 2.6	239
400	200	CP → FT	15.0 ± 2.3	206
400	300	FT + CP	18.3 ± 3.5	251
400	300	FT → CP	18.8 ± 2.5‡	250
400	300	CP → FT	22.3 ± 1.9	305
600	300	FT + CP	10.0 ± 0.7	137
600	300	FT → CP	14.0 ± 2.6	192
600	300	CP → FT	11.3 ± 0.4	155

*FT + CP = simultaneous treatment on Day 4; FT → CP = FT on Day 4 and CP on Day 5; CP → FT = CP on Day 4 and FT on Day 5.

†Survival of saline-treated mice was 7.3 ± 0.2 days. Each group had 7 mice. Survival times of mice treated with same dose of FT and CP were statistically equal. Mice treated with 600 mg/kg of FT survived a significantly shorter time than those treated with the other dose combinations.

‡A 45-day survivor for this treatment was not included in mean.

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Ultrasonic Hyperthermia and Drugs as Therapy for Human Tumor Xenografts[1,2]

Nagabhusan Senapati,* David Houchens, Artemio Ovejera, Ralph Beard, and Ronald Nines[3]

Ultrasonic energy can generate controlled, local hyperthermia (42°C–43°C), and ultrasonic hyperthermia results in increased cytotoxicity of chemotherapeutic drugs for the treatment of cancer. Human breast (MX-1) and lung (LX-1) tumors implanted subcutaneously in athymic nude mice were treated with a single application of ultrasonic (670 kHz, peak intensity of 5 watts/cm^2) hyperthermia (43.0°C ± 0.5°C) for 30 minutes in combination with suboptimal doses of cyclophosphamide, melphalan, or procarbazine. Delays in the tumor growth rate and temporary tumor regression were observed. Comparison of the tumor growth rate with single modalities, ie, drug or hyperthermia alone, shows evidence of synergistic effects of the combination of ultrasonic hyperthermia and melphalan on MX-1 and of hyperthermia and procarbazine on LX-1 tumors. [Cancer Treat Rep 66:1635–1639, 1982]

The study of the therapeutic effects of heat on malignant rodent tumors dates back to the late 1920s (1). During the 1970s, controlled hyperthermia (42°C–44°C) was beginning to be recognized as a very promising antitumor agent (2,3). In recent years there have been many encouraging studies on the use of hyperthermia alone and as an adjuvant to chemotherapy and radiotherapy against various types of malignancies (4,5).

The athymic mouse is an excellent animal model for the study of chemotherapeutic agents on human tumor xenografts (6–9) in vivo and additionally can serve as a model for combined modality therapy (10). Giovanella et al (11) have reported the use of microwave-induced hyperthermia in the nude mouse/human tumor xenograft model.

Human breast (MX-1) and lung (LX-1) tumors implanted subcutaneously in athymic mice were treated with ultrasonic hyperthermia in combination with cyclophosphamide, melphalan, or procarbazine. The animal model, the methods of ultrasonic hyperthermia treatment, and the results of these studies are presented in this paper.

MATERIALS AND METHODS

Female nude mice (6–8 weeks of age) with a randombred National Institutes of Health Swiss background were supplied by the Animal Genetics and Production Branch, National Cancer Institute (NCI), Bethesda, MD.

The human tumors used in this study were MX-1 (a mammary duct cell carcinoma) and LX-1 (established from a small [oat] cell lung carcinoma). These tumors are established for chemotherapy protocol testing as a part of the preclinical tumor panel of the Division of Cancer Treatment of the NCI (12). Protocols for testing were those established by the NCI and briefly were as follows.

Tumor fragments (3-mm^3) were implanted subcutaneously in the right subaxillary region, and the tumor volume, in cubic millimeters, was calculated for an ellipsoid by the formula $V = (a^2 \times b)/2$, where a is the width and b is the length in millimeters. This can be considered a valid estimation of weight in milligrams, assuming unit density. The tumor sizes were standardized in the different groups by obtaining relative tumor weight (RW) calculated by the formula RW = Wi/Wo, where Wi is the mean tumor weight at any given time and Wo is the mean initial tumor weight at the start of treatment. For evaluation purposes, any treatment group with a relative tumor size $\leq 42\%$ of that in the untreated control group on any day was considered to be responsive to therapy (13), and a relative size $< 1\%$ indicated tumor regression.

Melphalan, cyclophosphamide, and procarbazine were supplied by the Drug Evaluation Branch of the NCI. All were dissolved in physiologic saline and administered on schedules as indicated in the results section. For all animals receiving both chemotherapy and hyperthermia treatment, the drugs were administered 1 hour prior to ultrasound treatment. Doses injected were selected on the basis of experimental data from this laboratory and from the NCI.

The tumor xenografts were treated by a weakly focused beam of ultrasound. The ultrasonic energy was partially absorbed in the tumor and the internal temperature of the tumor was controlled at 43.0°C ± 0.5°C. The ultrasonic energy was produced by a 1-inch-thick lead zirconate titanate disk transducer with fundamental thickness

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[1] Received Dec 28, 1981; revised Mar 30, 1982; accepted May 10, 1982.

[2] Supported in part by contract N01-CM-67099 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Battelle Memorial Institute, Columbus, OH.

**Reprint requests to:* Nagabhusan Senapati, PhD, Ultrasonic Projects, Applied Dynamics and Acoustics, Battelle Memorial Institute, Columbus Laboratories, 505 King Ave, Columbus, OH 43201.

mode resonance at 670 kHz at the operating conditions. This transducer was carefully designed to deliver a maximum of 10 w of ultrasonic power without significant drifts or internal heating.

The ultrasonic output of the transducer was measured and calibrated by a radiation pressure gauge. At a total output of 5 w from the transducer, the axisymmetric intensity profile was measured at the focal plane by the transient thermocouple technique as shown in figure 1.

The schematic experimental setup for ultrasonic hyperthermia is shown in figure 2. The ultrasonic transducer was driven by a power oscillator with automatic gain control, and the transducer was coupled to the power oscillator by a transducer impedance matching network to maximize the load power factor and to improve the efficiency of the overall system. The power delivered to the transducer was continuously monitored by a radiofrequency directional power meter. The tumor temperature was continuously measured by a 30-gauge hypodermic needle type thermocouple positioned at a known location in the tumor. The core temperature of the tumor was controlled at 43.0°C ± 0.5°C by a temperature feedback circuit connected between the thermocouple implanted in the tumor and the power oscillator with automatic gain control.

The anesthetized nude mice were placed on a stainless steel platform with a 20-mm-diameter hole and an ultrasonic absorber placed beneath it. During insonation, the tumor, moistened with light mineral oil, was positioned on the top of the ultrasonic absorber. This design eliminates any possibility of undesirable standing waves of ultrasound in the tumor. Therefore, the ultrasonic dose levels could be precisely controlled and measured. During a typical experiment the tumor, rectal, and base temperatures were continuously monitored by a three-channel digital thermocouple readout device.

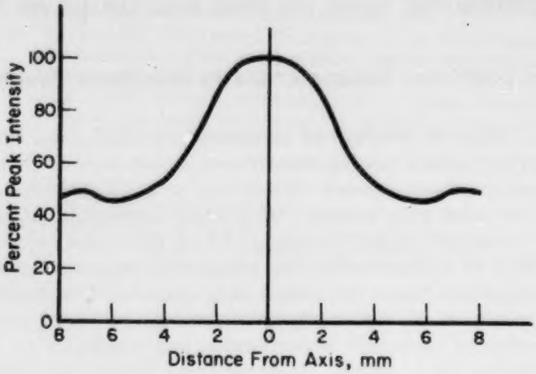

FIGURE 1.—Ultrasonic intensity distribution in water from weakly focused transducer at focal plane.

RESULTS

The measured temperature at different points inside any single tumor was observed to be within ± 0.5°C. The

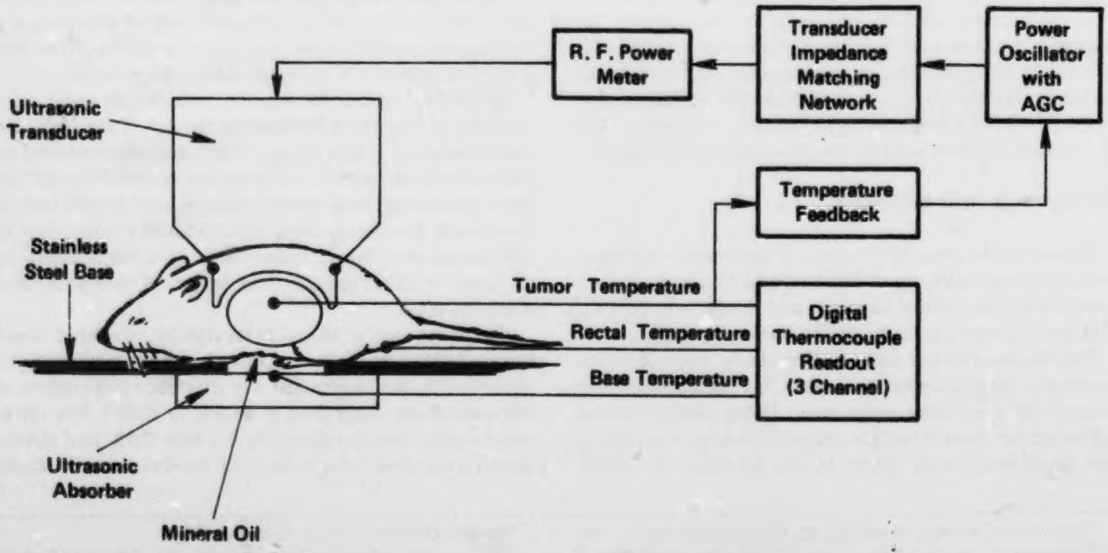
R. F. Power Meter
Transducer Impedance Matching Network
Power Oscillator with AGC
Ultrasonic Transducer
Temperature Feedback
Stainless Steel Base
Tumor Temperature
Rectal Temperature
Base Temperature
Digital Thermocouple Readout (3 Channel)
Ultrasonic Absorber
Mineral Oil

FIGURE 2.—Schematic of experimental ultrasonic hyperthermia setup.

desired temperature (43.0°C ± 0.5°C) was usually reached within 3–5 minutes from the starting time. The rectal temperature of the anesthetized mice was constant within 1°C during ultrasonic insonation (range, 25°C–27°C). This temperature is about 12°C below the normal rectal temperatures (37°C–39°C) of nude mice.

The results of the experiments are seen in table 1 and figure 3.

In experiment 1, the relative tumor size of the group that received both ultrasound and cyclophosphamide was lower on every measurement day than with either modality alone, indicating a retardation in growth, although the lowest T/C achieved was only 59%.

In experiment 2, the group receiving both melphalan and ultrasound had a marked retardation in tumor growth (T/C = 17%) for approximately 12 days, while the individual treatment modalities produced only marginal effects. The tumors in mice in the combination treatment group ultimately commenced regrowth. In an effort to establish a prolonged synergistic effect, a dose of 6 mg/kg/injection of melphalan was used in experiment 3. Marked tumor regression was effected in both the chemotherapy treatment group and the combination treatment group and no differences were seen between the two groups. To determine the effects of additional chemotherapy and hyperthermia, melphalan was given every 4 days for four injections and ultrasound was given on the days of the first and third chemotherapy treatments (experiment 4). The group receiving chemotherapy alone had a lower T/C value (28%) than the group receiving chemotherapy only in experiment 2. The group receiving two ultrasound treatments and the group receiving combination treatment had no differences in T/C values from those in experiment 2.

The LX-1 lung tumor was implanted, grown, and treated with procarbazine (experiment 5; fig 3). Procarbazine alone was not effective, while the hyperthermia treatment alone was minimally effective (T/C = 41%). The combination treatment produced marked tumor regression (relative tumor size, 0.46; T/C = 25%). The tumors in this group ultimately regrew. To determine if increased ultrasound treatment would produce more prolonged tumor regression, two treatments of ultrasound were given in experiment 6. In this experiment, the procarbazine produced some tumor retardation; the ultrasound treatment alone was effective in producing tumor retardation (T/C = 24%), while the combination treatment group had tumor regression (relative tumor size, 0.34; T/C = 9%). Ultimately, the tumors in the combination treatment group commenced regrowth at the same time as those in experiment 5.

Table 1.—Effects of ultrasonic insonation and/or chemotherapy on human tumor xenografts in nude mice

Experiment No.	Tumor[*]	Drug[†]	Dose (mg/kg/injection)	Drug schedule (Day)	Ultrasound schedule (Day)[‡]	Relative tumor size[§]	Optimal T/C[¶] (%)
1	MX-1	Cyclophosphamide	50	20	—	3.3	85
		—	—	—	20	3.5	90
		Cyclophosphamide	50	20	20	3.3	59
2	MX-1	Melphalan	4	20,24,28	—	3.9	49
		—	—	—	20	3.6	45
		Melphalan	4	20,24,28	20	1.3	17
3	MX-1	Melphalan	6	21,25,29	—	0.32	5
		—	—	—	21	1.9	53
		Melphalan	6	21,25,29	21	0.43	6
4	MX-1	Melphalan	4	20,24,28,32	—	2.6	28
		—	—	—	20,28	1.3	58
		Melphalan	4	20,24,28,32	20,28	1.4	14
5	LX-1	Procarbazine	100	22,26,30	—	0.97	47
		—	—	—	22	0.86	41
		Procarbazine	100	22,26,30	22	0.46	25
6	LX-1	Procarbazine	100	18,22,26	—	1.20	31
		—	—	—	18,26	0.88	24
		Procarbazine	100	18,22,26	18,26	0.34	9

[*] 3-mm^3 tumor fragments were implanted subcutaneously in right subaxillary region.

[†] Drugs were prepared in physiologic saline and were administered 1 hr before ultrasonic treatment except in experiment 1, in which cyclophosphamide was given 1 hr after hyperthermia.

[‡] An ultrasound transducer was used as described in the text to produce tumor temperatures of 43°C ± 0.5°C for 30 mins in each mouse.

[§] Relative tumor size = ratio of mean tumor weight of a group on evaluation day to mean tumor weight at initiation of treatment.

[¶] Optimal T/C (%) = lowest relative tumor size of treated group (T) as a % of relative tumor size of control group (C). T/C < 42% indicates response to therapy (13).

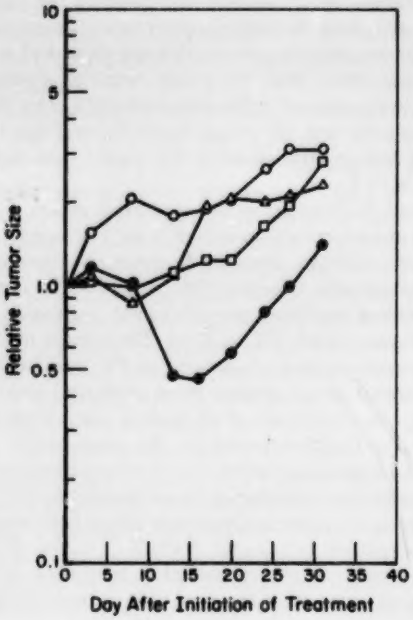

FIGURE 3.—Effect of ultrasonic hyperthermia and/or procarbazine (Pro) on LX-1 lung tumor xenografts. ○ = untreated mice (8 mice), Δ = ultrasonic insonation (5 mice, 30 mins/mouse), □ = Pro (7 mice; 100 mg/kg/; Days 22, 26, and 30 after tumor implantation), ● = ultrasonic insonation (5 mice, 30 mins/mouse) + Pro (100 mg/kg; Days 22, 26, and 30; 1st treatment 1 hr before ultrasound).

DISCUSSION

The results presented in this study demonstrated the usefulness of ultrasonic hyperthermia to treat human tumor xenografts in athymic mouse models in combination with suboptimal doses of chemotherapeutic drugs. This reduction of drug dose to achieve the same therapeutic effect is desirable. Therefore, ultrasonic hyperthermia would aid in the alleviation of undesirable toxic effects and at the same time maintain maximum efficacy.

Numerous studies to date have demonstrated significant therapeutic effects of heat in the treatment of cancer. Our research shows considerable promise for ultrasonic hyperthermia in combination with chemotherapy in the treatment of human tumors and it demonstrates the usefulness of this model for evaluation purposes. Some of the key advantages of ultrasonic hyperthermia as an adjuvant to chemotherapy are as follows:

(*a*) As shown in our results, the combination of ultrasonic hyperthermia and drug therapy has potential for synergistic effects. The mechanisms are believed to be "accelerated reaction rates" due to ultrasonic and thermal focusing of drugs.

(*b*) The heating volume and the temperature can be controlled within ± 0.5°C to conform to the shape and size of the tumor.

(*c*) During microwave and radiofrequency irradiation there is an induced artifact in the temperature-measuring process due to the interaction between the electromagnetic field and the temperature sensor. Because ultrasound is a form of acoustic energy, there is no electromagnetic artifact in the temperature measurement process.

(*d*) Ultrasound is a nonionizing radiation. To date we have found no confirmed publication in the literature on specific immunologic effects or changes in the mitotic index associated with ultrasonic exposure.

(*e*) Ultrasound is transmitted very poorly by air; hence, there is virtually no danger of exposing the operator or subject to hazardous levels of radiation. However, due to poor transmission of ultrasound in the air a coupling medium is required between the applicator and the tumor. In the present study, mineral oil has been found to be an effective medium.

The controllability, simplicity, and safety aspects of ultrasonic hyperthermia make it a promising system, and it has the advantage of treating human tumors in a proven animal model to assess the chemotherapeutic effects.

We believe this combination of ultrasonic hyperthermia and chemotherapy has the potential to achieve tumor regression at reduced dose levels and to reduce the associated side effects of the drugs in the treatment of cancer. Further development of dose, schedule, and treatment in such a model is desirable.

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Potential Cardiotoxicity With Mitoxantrone[1,2]

Frank C. Schell,* Hwee-Yong Yap, George Blumenschein, Manuel Valdivieso, and Gerald Bodey[3]

Mitoxantrone is a promising new agent developed in an attempt to find drugs with a broad spectrum of antitumor activity and devoid of cardiotoxicity. In a phase II clinical trial for refractory metastatic breast cancer, we observed congestive heart failure in four of 31 high-risk patients either during or after treatment with this drug. This report calls attention to that observation, and recommendation is made that further evaluation of this agent include careful cardiac monitoring. [Cancer Treat Rep 66:1641–1643, 1982]

Cardiotoxicity is a well-known and well-studied entity seen with several cytotoxic drugs, especially anthracyclines. It has been established that beyond certain dose levels a cardiomyopathy characterized by decreased myocardial contractility and clinical congestive heart failure will develop in a certain percent of patients treated with doxorubicin or daunorubicin (1–3).

Mitoxantrone is a new anthraquinone derivative synthesized in an attempt to develop an agent with a broad spectrum of antitumor activity and free of cardiotoxicity (4–5). Mitoxantrone showed significant activity against a number of animal tumor systems during preclinical studies (5). Available animal toxicology studies showed less cardiotoxicity than doxorubicin (6). In a recent phase II study of mitoxantrone for metastatic carcinoma of the breast refractory to conventional combination chemotherapy, we observed several patients who developed evidence of clinical congestive heart failure during or following therapy with this drug. This report calls attention to that observation and alerts the medical community to the necessity for careful and frequent cardiac evaluation in patients undergoing mitoxantrone therapy.

Thirty-one patients with refractory metastatic breast carcinoma were entered in this study. All patients had previously failed conventional combination chemotherapy, and all had previously received doxorubicin. The starting dose of mitoxantrone was 4 mg/m^2/day for 5 consecutive days, with courses repeated at 4-week intervals. During the study, four patients developed symptoms of congestive heart failure.

CASE REPORTS

Patient 1

Patient 1 was a 63-year-old white female whose breast cancer had been diagnosed 10 years previously. She had previously been treated with 5-FU, doxorubicin, cyclophosphamide, methotrexate, and tamoxifen for recurrent disease, with excellent responses. Prior doxorubicin was given to a total dose of 450 mg/m^2 and was discontinued 4 years prior to entry in the study. She was treated with mitoxantrone at a dose of 4 mg/m^2/day × 5 and achieved a complete remission after five courses. After seven courses, she developed clinical congestive heart failure with cardiomegaly, dyspnea, lower extremity edema, tachycardia, and bibasilar pulmonary rales. Chest X-ray showed evidence of congestive heart failure, ECG showed sinus tachycardia with nonspecific ST-T wave changes, and cardiac scan showed generalized hypokinesis and an ejection fraction of 0.46. She was treated with diuretics and digoxin and responded rapidly. There was no prior history of cardiovascular disease.

Patient 2

Patient 2 was a 65-year-old female who had had left mastectomy and postoperative radiation therapy 6 years previously. Prior to entry in the study, the patient was treated with 5-FU, doxorubicin, cyclophosphamide, and tamoxifen for recurrent disease, achieving a partial re-

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[1] Received Sept 18, 1981; revised Mar 29, 1982; accepted Apr 13, 1982.

[2] Supported in part by contract NO1-CM-97277 from the division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Medicine-Medical Breast Service and Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

*Reprint requests to: Dr. Frank Schell, Department of Medicine-Medical Breast Service, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

mission. Doxorubicin was discontinued 3 years prior to entry in the study after a total dose of 450 mg/m^2. When the patient again developed progressive disease, she was treated with mitoxantrone at a dose of 4 mg/m^2/day × 5. Due to severe myelosuppression, she required dose reduction on each of three subsequent courses. Following the fourth course of therapy she developed progressive disease and was switched to megestrol acetate. Approximately 2 months later, the patient was admitted to the hospital with acute pulmonary edema and died within 72 hours. This patient had no evidence of previous cardiovascular disease, and there were no known precipitation factors for the patient's heart failure. ECG showed marked sinus tachycardia and was otherwise unremarkable.

Patient 3

Patient 3 was a 72-year-old female who underwent mastectomy 17 years prior to entry in the study. Following 6 years of hormonal manipulations for recurrent disease, the patient began chemotherapy with 5-FU, doxorubicin, cyclophosphamide, and *Corynebacterium parvum*. Doxorubicin was discontinued 3 years prior to entry in the study after a total dose of 450 mg/m^2. Following 2 additional years of multiple chemotherapeutic agents, the patient was treated with mitoxantrone at 3.5 mg/m^2/day × 5. After four courses, she developed clinical congestive heart failure documented by physical examination and chest X-ray. ECG showed sinus tachycardia with nonspecific ST-T wave changes and cardiac scan showed generalized hypokinesis and an ejection fraction of 0.39. She responded to diuretics and digoxin. The patient's past history was significant only for mild hypertension of approximately 10 years' duration, which had been well-controlled with a low-sodium diet.

Patient 4

Patient 4 was a 31-year-old female who had had mastectomy 2$^1/_2$ years previously. She had been treated with adjuvant chemotherapy including 5-FU, doxorubicin, and cyclophosphamide. Doxorubicin was discontinued after a total dose of 300 mg/m^2. One year prior to entry in the study, recurrent disease was treated with a combination of doxorubicin, mitomycin, and dibromodulcitol. After a total dose of 500 mg/m^2 of doxorubicin, she developed clinical congestive heart failure and was noted to have decreased voltage on ECG and an abnormal ejection fraction on echocardiogram. She responded well to management with digoxin and diuretics, and therapy was switched to mitoxantrone. She received 4 mg/m^2/day × 5 every 21 days for a total of nine courses, achieving a partial remission. At that time, she developed recurring symptoms of congestive heart failure documented by chest X-rays and was found to have significant hypokinesis and decreased ejection fraction documented on both serial echocardiograms and nuclear scans.

DISCUSSION

Since this study was designed to evaluate the antitumor activity of this agent and not specifically to study potential cardiotoxicity, not all patients had serial cardiac evaluation during treatment.

Nevertheless, clinical congestive heart failure was observed in four of 31 patients, which was surprising and in excess of what we would normally expect to observe in a similar population of patients in a phase II drug study. In two recent phase II studies at our institution, 77 patients taken from the same population of refractory breast cancer patients had no incidence of congestive heart failure (7,8).

Whether, in fact, congestive heart failure was directly related to the drug cannot be determined from these patients. Certainly, this population of patients carries multiple risk factors for cardiovascular decompensation, including pretreatment with doxorubicin, precordial radiation therapy, and generally poor performance status. However, that does not alter the observation that this drug is potentially cardiotoxic, whether directly or possibly synergistically with doxorubicin. Therefore, studies evaluating mitoxantrone should include careful monitoring of cardiac function to determine the incidence and severity of any possible drug-related cardiotoxicity. Although the potential for myocardial damage may exist, especially in the face of previous treatment with maximal doses of doxorubicin, mitoxantrone has shown extremely encouraging evidence of activity in early studies, and we believe that continued evaluation is certainly warranted. In 27 evaluable patients, we observed one complete and five partial remissions (9). We hope that careful study will show that even if some evidence of cardiotoxicity does exist, it will be less severe than with doxorubicin, therefore making this drug a significant addition to the cytotoxic armamentarium.

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Combination Chemotherapy With 5-FU, 1-(Aminomethyl)-3-(2-chloroethyl)-3-nitrosourea (ACNU), and Mitomycin (FUM) for Carcinoma of the Pancreas[1]

Sadayasu Meguro,[2,*] Yasunobu Kuraishi,[2] Kenji Ichiba,[2] Masakazu Abe,[2] Tadahiro Yoda,[3] and Shigemaru Mikouchi[3]

In spite of the importance of pancreatic carcinoma, only a few chemotherapy combinations have undergone adequate trial. The combination of 5-FU, doxorubicin, and mitomycin (FAM) has been somewhat effective (1,2). Fujimoto et al (3) showed a distinct synergistic effect, "complementary inhibition" of DNA formation, between antimetabolites and 1-(aminomethyl)-3-(2-chloroethyl)-3-nitrosourea (ACNU), a newly developed water-soluble nitrosourea active against some experimental tumor systems. Based on this experiment and the enhanced therapeutic effect of a combination of mitomycin with 5-FU, we developed a combination consisting of 5-FU instead of 6-thioguanine as a purine analog, ACNU, and mitomycin (FUM), which has been evaluated in various advanced carcinomas of the gastrointestinal tract (4).

Between December 1978 and October 1981, 13 patients who had histologically documented metastatic pancreatic carcinoma with various measurable indicator lesions were treated with this protocol (table 1). An oral dose of 5-FU (200 mg) was given daily, combined with iv bolus injection of ACNU (70 mg/m^2) and mitomycin (10 mg/m^2). Chemotherapy was given until the wbc count was $< 2000/mm^3$ and/or the platelet count was $< 75{,}000/mm^3$, usually 14–28 days, depending on the individual bone marrow reserve. The average total dose of 5-FU was 4.2 g in each FUM course, and there was no significant difference between the dose received by responders and nonresponders. A resting period of 2–4 weeks was generally required. The whole course of chemotherapy was repeated every 6–8 weeks when the wbc count had returned to $3000/mm^3$

TABLE 1.—Characteristics of 13 patients

Patient No.	Age (yrs)	Sex	Karnofsky performance status (%)	Metastatic sites	No. of courses of FUM	Response	Response duration (mos)*	Survival duration (mos)†
1	62	M	80	Liver	2	None	—	4.5
2	35	M	30	Liver	1	None	—	4
3	70	F	40	Liver, peritoneum	4	Partial‡	8§	10¶
4	61	M	100	Liver	4	Partial‡	6	8¶
5	63	F	100	Liver	4	Partial‡	8§	9¶
6	69	F	90	Liver	3	Partial‡	4.5	5
7	55	M	80	Liver	2	None	—	5
8	70	M	40	Liver	1	None	—	5
9	55	F	30	Liver, peritoneum	1	None	—	2
10	59	M	40	Liver, peritoneum	2	Partial‡	4§	6¶
11	55	M	40	Liver, peritoneum	2	None	—	3.5
12	57	F	40	Liver	2	None	—	5
13	68	M	50	Liver, lung	5	Partial‡	12§	13¶

*From the day of 50% tumor reduction until disease progression.
†From Day 1 of chemotherapy.
‡Liver metastasis responded, but patients 3, 10, and 13 had stable disease in peritoneum or lung.
§Patient still responding.
¶Patient still alive.

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[1] Received Nov 9, 1981; revised Apr 7, 1982; accepted Apr 19, 1982.

[2] Department of Internal Medicine, Jikeikai University School of Medicine, Nishi-shinbashi, Minato-ku, Tokyo, Japan.

[3] Department of Surgery, Hibiya Hospital, Uchisaiwai-cho, Chiyoda-ku, Tokyo, Japan.

[*] *Reprint requests to:* Sadayasu Meguro, MD, Department of Internal Medicine, Jikeikai University School of Medicine, 3-25-8, Nishi-shinbashi, Minato-ku, Tokyo, Japan.

and the platelet count to 100,000/mm^3. Response to chemotherapy was evaluated by previously defined criteria (2).

Six of the 13 patients (46%) responded to FUM therapy. All 13 patients had dose-limiting hematologic toxic reactions. The median lowest wbc count per course was 1200/mm^3 (range, 800–2100/mm^3) and occurred by Day 19 (range, Day 13–30), with recovery by Day 31 (range, Day 14–58). The median lowest platelet count was 41,000/mm^3 (range, 17,000–78,000/mm^3) by Day 22 (range, Day 18–29), with recovery by Day 23 (range, Day 14–35).

There has been one drug-related death due to marked leukopenia among 41 patients, including the 13 in this study, who received FUM therapy for carcinomas of the gastrointestinal tract (4). Because of this and an effectiveness equal to that of the FAM combination, we believe that FUM chemotherapy may be a promising treatment for advanced pancreatic carcinoma. A further trial in a large number of patients is warranted to determine the effectiveness of FUM therapy.

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BRIEF REPORTS

Combination Carmustine (BCNU) and Dianhydrogalactitol in the Treatment of Primary Brain Tumors Recurring After Irradiation[1]

Robert T. Eagan,* Robert P. Dinapoli, Robert C. Hermann, Jr, Robert V. Groover, Donald D. Layton, Jr, and Mark Scott[2]

The nitrosoureas have historically been the most active class of agents in the treatment of recurrent primary brain tumors (1). Of these drugs, carmustine (BCNU) appears to be the most active (2). This activity can also be demonstrated when BCNU is used with postoperative irradiation and the results are compared to those of irradiation alone (3). In a similar situation, the combination of dianhydrogalactitol (DAG) with postoperative irradiation was shown to prolong median survival as compared to that after irradiation alone (4). Combination chemotherapy containing DAG has also shown antitumor activity in recurrent brain tumors (5). The combination of BCNU and DAG has been shown to be synergistic in the Glioma 26 experimental system (6). Consequently, a phase II evaluation of the combination was conducted.

MATERIALS AND METHODS

Forty-nine patients were entered in the study, 39 of whom had histologically confirmed brain tumors. Rebiopsy was not done at recurrence, and all tumor grading was based on the original surgery. One patient was evaluable for survival only (did not return for re-evaluation after initial treatment) and five patients were ineligible (one because of prior nitrosourea therapy, two who did not have primary brain tumors, and two who had no indicator disease on computerized tomographic [CT] brain scan).

Among the 43 patients evaluable for response and survival, the median age was 48 years (range, 22–74); 27 were males and 16 were females; and 14 had an Eastern Cooperative Oncology Group (ECOG) performance score (PS) of 1, 16 had a PS of 2, and 13 had a PS of 3–4. Disease progression was evident in 27 patients in < 1 year from diagnosis and in 16 after ≥ 1 year. Median time from diagnosis to start of chemotherapy was 30.5 weeks. One patient had had prior chemotherapy (aziridinylbenzoquinone).

Tumor types and patient characteristics are shown in table 1. All patients had progressive disease (determined either neurologically and/or by CT brain scan—usually both) at entry in the study. Eligibility requirements included a wbc count ≥ 3500/mm^3, a platelet count ≥ 100,000/mm^3, a serum creatinine ≤ 1.5 mg/dl, a contrast-enhancing tumor mass on CT brain scan, and signed informed consent by the patient or the responsible next of kin. No patient could have received prior chemotherapy with a nitrosourea or a hexitol, primary chemotherapy with any other agent within 4 weeks, or radiation therapy within 8 weeks (unless disease progression occurred during radiation therapy—as it did in three patients).

A regression required a definite decrease in the size of the contrast-enhancing lesion on CT brain scan (not edema) coupled with neurologic improvement, provided that the latter symptoms were related to the tumor and not to surgery. Disease progression was defined as a definite increase in tumor size on CT brain scan or no change in tumor size but neurologic deterioration over two treatment cycles. Stable disease was anything else. Response determinations were made with patients receiving no corticosteroids or stable or tapering doses of corticosteroids. A neurologic examination was performed by a neurologist prior to each planned treatment. CT brain scans were done prior to the planned second treatment and then before every third subsequent treatment if regression was noted, or prior to the third planned treatment and then before every third subsequent treatment if stability was noted. Both evaluations were repeated at disease progression.

Treatment consisted of 90 mg/m^2 of BCNU given iv over 45–60 minutes plus 70 mg/m^2 of DAG given iv over 5 minutes. The first re-treatment was scheduled for 5 weeks, with subsequent re-treatments during Year 1 at 5–7-week intervals and during Year 2 at 10–12-week intervals. Treatment was discontinued at disease progression or at the end of 2 years. Drug dosages were adjusted

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[1] Received Jan 29, 1982; revised Mar 31, 1982; accepted May 6, 1982.

[2] Division of Medical Oncology (*R. T. Eagan*), Department of Neurology (*R. P. Dinapoli, R. C. Hermann, Jr, R. V. Groover,* and *D. D. Layton, Jr*), and the Section of Medical Research Statistics (*M. Scott*), Mayo Clinic, Rochester, MN.

**Reprint requests to:* Robert T. Eagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

TABLE 1.—Distribution of patients and regression rates for each category

Tumor types and strata	No. of patients— In study	No. of patients— With tumor regression
All evaluable patients	43	20 (46.5%)
Astrocytoma		
Grade 1	2	0 (0.0%)
Grade 2	13	5 (38.5%)
Grade 3	14	8 (57.1%)
Grade 4	3	1 (33.3%)
Oligodendroglioma grade 2	1	1 (100.0%)
Unbiopsied "glioma"	10	5 (50.0%)
Males	27	12 (40.9%)
Females	16	8 (50.0%)
Age		
$\leq$ 48 yrs	22	9 (41.7%)
> 48 yrs	21	11 (52.4%)
Diagnosis to disease progression		
< 1 yr	27	13 (48.1%)
$\geq$ 1 yr	16	7 (43.7%)
ECOG PS		
0–1	14	6 (42.8%)
2–4	29	14 (48.3%)

in an attempt to obtain wbc and platelet count nadirs < 4000 yet ≥ 2000 and $< 100,000$ yet $\geq 75,000$, respectively. Subsequent treatment was not repeated until the wbc count had increased to ≥ 2750 and the platelet count to $\geq 95,000$.

RESULTS

Tumor Response

Of the 43 evaluable patients, 20 (46.5%) had tumor regressions, 16 (37.2%) had a period of stability, and seven (16.3%) had immediate disease progression. Regression rates were not significantly different for tumor grade (1–2 vs 3–4 vs unknown), age ($\leq$ 48 vs > 48 years), sex, time from diagnosis to disease progression (< 1 vs $\geq$ 1 year), or ECOG PS (0–1 vs 2–4), with $P > 0.66$ in each chi-square test of equality of proportions.

Time to Disease Progression and Survival

Overall median time to disease progression was 3.6 months. Values for median time to disease progression for patients with regression, stability, and immediate disease progression were 5.8, 2.2, and 1.1 months, respectively. Overall median survival was 7.4 months. Median survival times for patients with regression, stability, and immediate disease progression were 12.7, 5.2, and 1.9 months, respectively. None of the covariates given above for response influenced time to disease progression or survival ($P > 0.05$ in each log-rank test).

Toxicity

The median number of courses received was three (range, one to 16). Only eight patients received a total BCNU dose of > 700 mg/m^2 (range, 725–1100). These eight patients received 11.25 courses on the average (range, eight to 16).

Nausea and vomiting were mild and infrequent. No renal or pulmonary toxicity was noted. Hematologic toxicity was mild. After the first treatment, only 30% of the patients experienced leukopenia (wbc count nadir < 4000/mm^3) and only 28% had thrombocytopenia (platelet count nadir < 100,000/mm^3). Considering all courses, 54% of the patients experienced leukopenia (only 2% of the nadirs were < 2000/mm^3) and 58% had thrombocytopenia (only 10% of the nadirs were < 50,000/mm^3). Among the eight patients mentioned above, 75% had wbc counts of 2500–3500 and normal platelet counts at 6 weeks after therapy for most of the latter half of their therapy, suggesting a slowed wbc recovery. One of the eight patients had a slight rise in the SGOT (1.5 times normal), which decreased by 20% in the 2 months following completion of treatment. No treatment-related deaths were noted.

DISCUSSION

The combination of BCNU and DAG was well-tolerated by patients with primary brain tumors recurrent after irradiation. Tumor regressions were noted in 46.5% of the patients treated. This regression rate appeared fairly constant regardless of age, sex, grade of tumor, time from initial diagnosis to disease progression, prior chemotherapy, and PS at the start of chemotherapy. When we reevaluated the data on BCNU alone presented by Wilson et al (7), trying to include comparable tumors (ie, astrocytomas, unbiopsied "glioma," etc) and similarly stringent response criteria (ie, CT or radionuclide scan improvement plus neurologic improvement in *all* eligible patients treated), we obtained a regression rate of 21.4% (six of 28 patients). We also evaluated our nonprotocol use of BCNU in patients with similar tumors and prior chemotherapy exposure (mainly DAG), using our usual response criteria reported here. We obtained a 12.5% (three of 24 patients) regression rate. Consequently, we believe a prospectively randomized trial of BCNU plus DAG versus BCNU alone should be done and that the doses of BCNU and DAG could be safely increased to 100 and 75 mg/m^2, respectively. The response data from this study suggest that patients with recurrent "low grade" glioma and unbiopsied "glioma" could be included in such a trial if proper stratification is employed.

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Phase II Trial of *m*-AMSA in Hepatocellular Carcinoma: A Southwest Oncology Group Study[1,2]

R. M. Bukowski,[3] S. Legha,[4] J. Saiki,[5] H.J. Eyre,[6] and R. O'Bryan[7,*]

Twenty-three patients with hepatocellular carcinoma were treated with *m*-AMSA at a dose of 120 mg/m^2 iv repeated at 4-week intervals. Toxicity was primarily hematologic. Partial responses occurred in two of 14 previously treated patients and in one of nine previously untreated patients. The overall activity of *m*-AMSA in patients with hepatocellular carcinoma appears minimal. [Cancer Treat Rep 66:1651–1652, 1982]

m-AMSA is one of the large family of amino acridines synthesized by Cain and Atwell (1). The drug was selected for further study because of a broad spectrum of experimental antitumor activity in a number of murine tumors(2). Phase I clinical trials of *m*-AMSA were initiated in 1977 and several dose schedules with acceptable toxicity were identified (3,4). These studies demonstrated leukopenia to be the major dose-limiting toxic effect. In 1978, the Southwest Oncology Group initiated a phase II study of *m*-AMSA in patients with hepatocellular carcinoma. The dose schedule employed was that previously reported by Von Hoff et al (3). The purpose of the present paper is to report this experience.

MATERIALS AND METHODS

Patients with histologically confirmed hepatocellular carcinoma not amenable to surgical resection were eligible for the study. All patients had measurable disease, and prior therapy with either chemotherapy or radiation was allowable. Patients were divided into good- and poor-risk status, with the former group defined by the following criteria: age $\leq$ 65 years, no prior radiotherapy, normal tolerance to prior chemotherapy (if any), and bilirubin < 1.2 mg/100 ml. *m*-AMSA was given at a dose of 120 mg/m^2 in good-risk patients and 90 mg/m^2 in poor-risk patients. In patients with markedly abnormal liver function (bilirubin $\geq$ 3.0 mg/100 ml and/or SGOT greater than three times normal), the starting dose was 60 mg/m^2. *m*-AMSA was administered iv in 500 ml of dextrose in water over 1 hour. Courses were repeated at 4-week intervals, and dose adjustments for subsequent courses were based on nadir blood cell counts. Dose levels were: 40, 60, 90, 120, 140, 160, and 180 mg/m^2. Doses were altered as follows: if wbc count $\geq$ 3000/mm^3 and/or platelet count $\geq$ 100,000/mm^3, increase by one dose level; if wbc count < 2000/mm^3 and/or platelet count < 75,000/mm^3, decrease by two dose levels; and if intermediate nadir counts, no change. Cbc with platelet count, LDH, SGOT, alkaline phosphatase, and bilirubin were obtained before treatment, on Day 10 of each course, and before each subsequent course. Lesions were measured prior to each course, and BUN and creatinine readings were obtained prior to each treatment.

An adequate trial of therapy consisted of one course of *m*-AMSA with subsequent followup of 4 weeks. Standard Southwest Oncology Group response criteria were employed.

Twenty-five of 27 registered patients were eligible (the ineligible patients had metastatic adenocarcinoma of unknown primary and cholangiocarcinoma). Twenty-three patients were evaluable for response and toxicity, with the two inevaluable patients never receiving *m*-AMSA.

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[1] Received Oct 19, 1981; revised Apr 15, 1982; accepted Apr 21, 1982.

[2] Supported in part by Public Health Service grants CA-12213, CA-13238, CA-04915, CA-04919, CA-03096, CA-14028, CA-22411, CA-03389, CA-04920, CA-26766, CA-22433, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Cleveland Clinic Foundation, OH.

[4] The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[5] University of New Mexico School of Medicine, Albuquerque.

[6] University of Utah Medical Center, Salt Lake City.

[7] Henry Ford Hospital, Detroit, MI.

[*] *Reprint requests to:* Southwest Oncology Group (SWOG-7920), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

RESULTS

Clinical and response data for the 23 evaluable patients are outlined in table 1. Most patients had received previous chemotherapy, and 20 had measurable disease in the liver. Most patients were symptomatic, with 20 of 23 having a performance status of 1 or 2. The median age of evaluable patients was 50 years (range, 15–68). Three patients responded, and all responses were partial. The median number of courses of *m*-AMSA administered was two (range, one to 16+). The responses observed were brief in two patients (1 and 2 mos) and lasted 18 months in a third patient. Two of the responders received 90 mg/m^2 of *m*-AMSA, and the third received 120 mg/m^2. Previous treatment status did not influence response, with two of 14 previously treated and one of nine previously untreated patients responding. The median survival was 22.5 weeks for previously untreated patients and 20 weeks for the previously treated group (Kaplan-Meier survival curves).

Toxicity was moderate and predominantly hematologic. Leukopenia ($\leq$ 3000 cells/mm^3) occurred in four of nine previously untreated and in nine of 14 previously treated patients. It was life-threatening (wbc count < 1000/mm^3) in two patients, and in one patient a drug-related death secondary to infection was recorded. Thrombocytopenia was milder, with only five of 23 patients having nadir platelet counts $\leq$ 100,000/mm^3. The presence of severe hematologic toxicity (wbc count < 2000/mm^3 or platelet count < 50,000/mm^3) was noted in four patients, two of whom had elevations of bilirubin ($\geq$ 1.5 mg/100 ml) prior to therapy. Nonhematologic toxic effects included: nausea and vomiting (three of 23 patients), pain with *m*-AMSA injection (four), mucositis (two), and chemical cellulitis (one). Liver function abnormalities occurred in five patients and consisted of transient elevations of liver enzymes (SGOT, SGPT, and LDH) or bilirubin. No cardiac toxicity was identified.

TABLE 1.—Patient data

Treatment status	No. of patients
No previous therapy	9
Previous chemotherapy	14
Hepatic artery infusion*	5
Iv 5-FU and doxorubicin with or without miscellaneous agents	9
Sex	
Male	14
Female	9
Dose levels (mg/m^2)	
60	6
90	11
120	6
Site of measurable disease	
Liver	
Hepatomegaly	12
Liver/spleen scan, computerized axial tomographic scan, liver	7
Lung	3
Lymph node	1
Bilirubin levels (mg/100 ml)	
< 1.0	13
1.0–1.5	5
> 1.5	5
Response data	
Complete	0
Partial	3
None	20

*Multiple regimens employed (1 patient each): 5-FU, 5-FU + mitomycin, 5-FU + doxorubicin, streptozocin + mitomycin + 5-FU, FUDR + streptozocin + doxorubicin.

CONCLUSIONS

Three partial responses (13%; 95% confidence interval, 2.8%–33.6%) and moderate hematologic toxic reactions were seen in this study. Falkson et al (5) have reported a phase II study employing *m*-AMSA at a dose of 120 mg/m^2 every 3–4 weeks. Thirty-five previously untreated patients were studied, with one partial response seen and a median survival of 13 weeks in the group. Overall, 71 patients with hepatomas have reportedly received *m*-AMSA, with five of 71 (7%) demonstrating objective responses. Goldsmith et al (6) reported a partial response in a single patient. Lightdale et al (7) described 12 patients with hepatomas treated at a similar dose schedule: no response was seen, but in three patients stable disease of varying periods was reported. It appears that *m*-AMSA produces occasional tumor regression in patients with hepatomas, but overall activity appears to be minimal.

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BRIEF REPORTS

Phase II Trial of Bisantrene for Advanced Hypernephroma[1,2]

Howard Scher, Simeon Schwartz, Alan Yagoda,* Robin Watson, and Leslie Faye[3]

Bisantrene, a new guanidino-substituted anthracene derivative, has recently been brought into phase I studies, and objective tumor regression was noted in patients with hypernephroma (1–3). In addition, Von Hoff et al (4,5) demonstrated significant antitumor activity against hypernephroma in the human tumor cloning system: four of 18 patients exhibited a 70% reduction in the number of colonies and nine of 18 experienced a 50% reduction. Thus, a phase II disease-oriented trial in patients with advanced hypernephroma was undertaken at Memorial Hospital.

MATERIALS AND METHODS

Twenty-seven patients with bidimensionally measurable, pathologically confirmed hypernephroma received bisantrene. All patients had a complete medical history and physical examination, with measurement of the longest perpendicular diameters of palpable lesions by two independent investigators. Prior to each dose, a cbc, SMA-12, 5′-nucleotidase, gamma-glutamyl transpeptidase, and chest X-ray were obtained. In selected cases, liver and bone radionuclide scans and computerized transaxial tomograms (CT scans) were performed and repeated every 6 weeks. All X-rays and scans were evaluated independently (R. C. Watson). Most patients obtained a cbc 7, 10, and 14 days after each dose. Patient entry required adequate hematologic parameters (wbc count > $4000/\text{mm}^3$; platelet count > $150{,}000/\text{mm}^3$), Karnofsky performance status > 50%, bilirubin < 2.0 mg/100 ml, BUN < 30 mg/100 ml, creatinine < 1.5 mg/100 ml, and bidimensionally measurable lesions. Prior hormonal therapy and cytotoxic drugs were discontinued at least 3 weeks prior to the first dose of bisantrene (nitrosoureas were stopped for 6 weeks), except for methotrexate and vinblastine which were stopped at 2 weeks. An adequate trial was defined as one dose with myelosuppression and/or rapid disease progression (> 50% increase in the size of measurable lesions), or two doses with tumor progression (> 25% increase in size) and a survival of 6 weeks. Response criteria included: *partial remission (PR)*—a > 50% decrease in the summed products of the perpendicular diameters of all measurable lesions for > 1 month; *minor response*—a 25%–49% decrease in measurable lesions for > 1 month; *stable disease*—a < 25% change in tumor size for 3 months; and *disease progression*—a > 25% increase.

The drug was administered iv every 3 weeks in a slow infusion over a minimum of $1^1/_2$ hours, with monitoring of blood pressures at 10-minute intervals. In all cases, the first dose was given to patients in the supine position. Lyophilized powder was diluted to a maximal concentration of 2.0 mg/ml (range, 0.1–2.0) and infused under protection from light within 2 hours of preparation. Initially, the recommended dose was 260 mg/m^2, but in the absence of significant myelotoxicity, the dose was increased to 280 mg/m^2. One patient who had malignant hepatomegaly was started at 220 mg/m^2. Dose escalation by 20 mg/m^2 increments was permitted: of 19 patients given 260 mg/m^2, 14 received 280 mg/m^2 and five received 300 mg/m^2; three of seven patients started at 280 mg/m^2 received 300 mg/m^2.

Patient Characteristics

There were 22 males and five females, with a median age of 56 years (range, 32–77) and a median performance status of 70% (range, 50%–90%). Seventeen (63%) patients had not received previous chemotherapy, while seven had received one drug and three had received more than three drugs (including anthracycline derivatives in four). Five patients had had prior irradiation, 14 had had hormonal therapy, and one had received immunotherapy. Median time from symptoms to diagnosis was 2 months (range, 0–5) and from diagnosis to protocol treatment was 8 months (range, 0–154). Primary indicator lesions were

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[1] Received Feb 8, 1982; revised Apr 22, 1982; accepted May 6, 1982.

[2] Supported in part by a grant from the American Cyanamid Co, Inc, and Public Health Service grant CA-05826 and contract N01-CM-07337 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Drs. Scher and Schwartz are the recipients of a Medical Oncology fellowship.

[3] Departments of Medicine (Solid Tumor Service) (*H. Scher, S. Schwartz, A. Yagoda,* and *L. Faye*) and Diagnostic Radiology (*R. Watson*), Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Alan Yagoda, MD, Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

in the lung (16 patients); subcutaneous areas (four); abdomen, as evaluated by CT scan (five); liver (one); and lymph nodes (one). Secondary parameters included pleural-based masses (two patients), palpable abdominal masses (five), abnomal CT scans (four), a subcutaneous mass (one), malignant hepatomegaly (one), and hypercalcemia (two).

RESULTS

There were no responses (95% confidence limits, 0–12%) in 26 adequately treated patients, 16 (95% confidence limits, 0–16%) of whom had had no prior cytotoxic chemotherapy. Two patients had stable disease for 6 and 3 months, respectively. One patient who died 10 days after the first dose was considered inadequately treated.

There were 64 doses: four patients had one dose; 12, two doses; seven, three doses; two, four doses; and one, seven doses. Although five of 23 patients evaluable for acute toxic reactions experienced no side effects, nausea was observed in five (21%), vomiting in six (26%), light-headedness in four (18%), myalgias in three (13%), fever in seven (30%), and chills in five (21%). Fever with myalgias and chills, which increased with subsequent courses, was partially blocked with diphenhydramine in three patients and completely ameliorated with corticosteroids in three. Discontinuation of corticosteroids resulted in a return of all symptoms in one patient. Four patients who were receiving concurrent corticosteroids for brain metastases or hypercalcemia experienced no side effects. One patient complained of severe shoulder pain in a contralateral arm during his third cycle which required analgesics, and another had hypotension during infusion of bisantrene which clearly was related to the rate of administration. Severe rigors and chills resulted in discontinuation of bisantrene during the third course in one patient. Local reactions including erythema and warmth at the iv site were common, and phlebitis was noted in seven patients, with significant arm swelling for 3–4 months in six. One patient given 5-FU in the same arm 2 months later had recurrence of his phlebitis. Two patients refused further therapy after developing such side effects. While no drug-related abnormalities in liver function tests were observed, one patient with extensive malignant hepatomegaly became encephalopathic 2 days after receiving bisantrene which resolved spontaneously 1 day later. There was no evidence of ECG changes or hepatic or renal dysfunction in three patients who received 1040 mg/m^2 (total, 2060 mg), 1120 mg/m^2 (2550 mg), and 1940 mg/m^2 (3608 mg), respectively.

Twenty-five patients were evaluable for hematologic toxicity. Myelosuppression was observed in 16 patients; leukopenia (< 4000 cells/mm^3) occurred in 16, and thrombocytopenia (< 150,000 cells/mm^3) occurred in four. One patient who had had no prior therapy and was given 260 mg/m^2 had dose-limiting thrombocytopenia. The wbc and platelet count nadirs were observed on Day 8, with recovery by Day 14. In 19 patients given 260 mg/m^2, nine (47%) had a wbc count < 4000/mm^3 and one (5%) had a wbc count < 2000/mm^3 (median nadir, 3400/mm^3; range, 1900–3800); two had thrombocytopenia (88,000 and 143,000 platelets/mm^3). In 19 evaluable patients given 280 mg/m^2, nine (43%) had a wbc count < 4000/mm^3 and two (17%) had a wbc count < 2000/mm^3 (median nadir, 2900/mm^3; range, 1800–3800); two had platelet counts < 150,000/mm^3. Three of five evaluable patients given 300 mg/m^2 had a wbc count < 4000/mm^3 (range, 2700–3200), but none had thrombocytopenia. No patient had significant drug-induced anemia.

DISCUSSION

Toxicity was similar to that previously reported (1–4) except that prolonged infusion of bisantrene eliminated drug-induced hypotension in all but one patient in whom blood pressure changes were clearly related to the rate of infusion. Arm swelling and chemical phlebitis were more disabling, lasting > 3 months and requiring discontinuation of the drug in two cases. Recent studies (American Cyanamid Co) have shown that crystallized bisantrene is deposited on the venous endothelium in rabbits, and both hypotension and chemical phlebitis may be ameliorated by decreasing the concentration and infusion rate of the drug. Flu-like symptoms could be prevented by a 2-day course of corticosteroids. No evidence of cardiac toxicity was noted in preclinical toxicology studies of bisantrene, and, of interest, four patients who had had prior anthracyclines and three given > 1000 mg/m^2 had no evidence of drug-induced cardiac abnormality.

In the present study, no responses occurred in 26 adequately treated patients, 16 of whom were not pretreated. In phase I trials, there was one PR for 6+ weeks among three patients given a daily dose, and one PR for 2+ months in a patient given 200 mg/m^2 (2,4). In a phase II trial with 260 mg/m^2 every 3 weeks, three of 32 patients achieved a PR.[4] Combining all results including those of the present study, only five of 62 (8%) patients achieved PR. Such poor results in vivo suggest that the in vitro human cloning assay system is, as yet, ineffective in predicting a drug's efficacy for hypernephroma. While a higher dose of bisantrene could be tried, in the dose and schedule recommended following phase I trials and used in the present study, the drug has demonstrated no efficacy against advanced hypernephroma. New drugs are still needed.

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BRIEF REPORTS

Therapeutic Effect of Dopamine Infusion on Human Malignant Melanoma[1,2]

Michael M. Wick[3,*]

Dopamine is a novel antitumor agent that has shown activity against B16 melanoma. Four patients with advanced malignant melanoma were infused with dopamine at 20 μg/kg/min for 48–120 hours. Tumor and bone marrow samples were obtained before and after treatment for comparison. Each of the patients tested showed significant reduction in the labeling index of tumor cells following treatment. These results suggest that dopamine is capable of attaining cytotoxic levels necessary to induce a biochemical inhibition of human malignant melanoma. [Cancer Treat Rep 66:1657–1659, 1982]

Levodopa and dopamine have been shown to be effective antitumor agents in several experimental tumor systems, including melanoma (1–3). Since these agents also play a central role in melanin biosynthesis, it was anticipated that they might be effective against melanocytic tumors that contain the oxidative enzyme tyrosinase. This hypothesis was supported experimentally, since cytotoxic effects, although qualitatively similar among the various cell lines, were generally quantitatively greater in cells possessing tyrosinase.

Preclinical studies have indicated that these drugs exert a specific biochemical effect on human tumor cells as reflected in the selective inhibition of DNA synthesis. Measurements of thymidine incorporation provide a sensitive and reproducible index of the biochemical effect of these drugs that correlates with antitumor activity (4). The inhibition of thymidine incorporation has also been shown to be an indicator of response to a variety of conventional chemotherapeutic agents (5).

Since there is currently no effective therapy for the treatment of advanced malignant melanoma in man, we wished to evaluate the significance of these observations in patients with advanced disease. We sought to determine if cytotoxic levels could be achieved using dopamine and if a biochemical inhibitory effect on tumor cells in vivo as measured by thymidine incorporation could be demonstrated as a prerequisite for an antitumor response.

MATERIALS AND METHODS

Patients and Treatment Program

Four patients with histologically proven metastatic malignant melanoma for whom no other treatment was available were accepted into the study. The treatment protocol had been approved for scientific content and human protection by the appropriate institutional review boards. Patients were premedicated with propranolol, 20 mg every 4 hours, and dopamine infusions were maintained at maximally tolerable levels for 48–120 hours with constant monitoring of cardiovascular parameters. Bone marrow aspirates were taken immediately prior to and 24 hours after the initiation of therapy and processed as described below for labeling and scintillation indices. Each patient had multiple cutaneous nodules, which permitted excisional biopsy of the tumor and determination of labeling index in the biopsy specimen before and 24 hours after infusion was completed. The plasma level of dopamine was determined by high-pressure liquid chromatography.

Labeling and Scintillation Indices

The method for determination of the scintillation index in bone marrow aspirates has been described (6). Labeling and scintillation indices of the tumors were determined by a modification of the techniques reported by Murphy

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[1] Received July 13, 1981; revised Apr 29, 1982; accepted May 6, 1982.

[2] Supported by Public Health Service grants CA-27128 and CA-19589 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by American Cancer Society grant CH-126.

[3] Division of Medicine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA.

[*] *Reprint requests to:* Dr. Michael M. Wick, Division of Medicine, Sidney Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

et al (5). Single cell suspensions were prepared from the tumor biopsy, and after filtration through a cotton plug, the cells were exposed to 2–10 microcuries of [^{3}H]TdR for 1 hour. An aliquot of the above suspension was cytocentrifuged and placed on microscope slides. The slides were then coated with Kodak photography emulsion NTB-2, exposed for 5 days in a dark box, and developed with G19 all-purpose fixer. Following Wright staining, the labeled cells were counted. Cells containing at least eight grains over the nucleus were counted as positive. A minimum of 1000 cells/specimen was counted and each sample was done in triplicate, with background always less than five grains per cell.

RESULTS

Toxicity

The major toxicity was related to cardiovascular effects of dopamine. All patients reported unpleasant feelings of a hyperdynamic state characterized by increased force of cardiac contractions and increased heart rate. Under no circumstances was the heart rate allowed to increase above 120 beats/minute for the duration of the infusion. Although blood pressure was kept below 150 mm systolic, it would occasionally rise above 180 mm systolic for brief periods. Patients 1 and 3 reported occipital headache during treatments. All side effects were reversible upon cessation of therapy, and each patient was discharged from the hospital 24 hours following completion of therapy. The initial plan was to re-treat patients at 1–2-week intervals; however, persistent fatigue, especially in the older patients, precluded re-treatment.

Treatment Results

Table 1 describes the results of treatment. At the maximally tolerated dose of 20 μg/kg/min, steady-state plasma concentrations between 10^{-5} M and 10^{-6} M were achieved. Comparison of the labeling index of the tumor, biopsied prior to the start of treatment and again 1 day after cessation of treatment, indicated a consistent tenfold reduction in labeling index. Bone marrow samples processed before and 24 hours after the start of infusion consistently showed a decrease in the incorporation of radioactively labeled nucleotide precursor. There was no change observed in the labeling index or in the peripheral wbc count.

DISCUSSION

Dopamine is a novel antitumor agent that has been used extensively in man for the treatment of vasomotor disorders. In the preliminary studies, the cardiovascular effects of dopamine were important in limiting the dose and potential use of dopamine as an antitumor agent. Concentrations $> 10^{-5}$ M could be achieved, which began to approximate the cytotoxic level estimated from in vitro experiments, and encouragingly, significant drops in the labeling index of tumor biopsy specimens were observed in each patient. The significance of this result is also reinforced by the simultaneous decrease in incorporation of radiolabeled nucleotide precursor into bone marrow cells. These observations are consistent with the results found in experimental leukemia models (7).

The reduction in labeling index of tumor cells, as compared to no effect upon labeling index of bone marrow cells, suggests that we might be exerting a selective biochemical inhibitory effect on tumor cells. However, the toxicity of dopamine prevented re-treatment, which might be necessary for the observation of tumor regression.

Our studies suggest that even with dopamine, one can achieve doses that approximate cytotoxic levels in humans with malignant melanoma but the dose is limited by potent vasomotor effects of the drug. With the advent of dopamine analogs, which are devoid of cardiovascular effects and can be administered at greatly increased doses, we are hopeful that this dose-limiting toxicity can be avoided. Specifically, the drug 3,4-dihydroxybenzylamine shows enhanced therapeutic activity in all experimental models, primarily through an increase in maximally tolerated dose (8).

TABLE 1.—Effect of dopamine on tumor and bone marrow in patients with malignant melanoma

Patient No.	Duration of treatment (hrs)	Plasma level (M)	Tumor (% labeling index)		Bone marrow*		
			Preinfusion	Postinfusion	TdR	CdR	UdR
1	120	4.0×10^{-5}	2.0	0.2	26	18	25
2	72	5.2×10^{-5}	3.0	0.2	25	20	25
3	48	3.5×10^{-5}	1.0	0.1	41	34	33
4	48	3.1×10^{-5}	3.0	0.2	17	25	35

*Values represent samples taken 24 hrs after infusion began and are expressed as a % of values obtained at start of infusion. Typical control values were TdR, 15,000; CdR, 800; and UdR, 1475 cpm/10^6 cells.

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BRIEF REPORTS

Platinum Concentrations in Human Glioblastoma Multiforme Following the Use of Cisplatin[1]

Eric M. Bonnem,[2,3,*] Charles L. Litterst,[4] and Frederick P. Smith[3]

A 24-year-old female with glioblastoma multiforme was not given a nitrosourea because of interstitial lung disease. Cisplatin was administered monthly for five cycles until disease progression. A second debulking procedure was performed and tumor tissue was assayed for the presence of platinum. Levels were measurable 7 weeks after the previous course of treatment. [Cancer Treat Rep 66:1661–1663, 1982]

The blood-brain barrier has been one of the major hindrances to the development of effective chemotherapy for malignant neoplasms of the central nervous system. Because of their high lipid solubility, the nitrosoureas have been the mainstay of treatment. Survival benefit with the use of nitrosoureas given subsequent to standard radiation therapy has been only marginal.

The recent introduction of cisplatin into clinical practice has augmented the capabilities of clinical oncologists to treat a variety of malignant tumors. Its levels are measurable in liver, lungs, kidneys, ovaries, and uterus (1–3). Distribution studies in man (4) and animals (1,2,5–8) have shown poor penetration into the central nervous system. However, studies of tumors implanted into the brains of animals have demonstrated the presence of platinum in the tumor tissue (9). Since the blood-brain barrier is violated at the time of implantation, it is difficult to extract from such studies any conclusions about platinum penetration into malignant neoplasms in man. The following is a case report of a patient with glioblastoma multiforme treated with cisplatin; concentrations of platinum were measured in the tumor 7 weeks following the last administered dose of cisplatin.

CASE REPORT

In October 1980, a grade IV glioblastoma multiforme was diagnosed in the parietotemporal area of a 24-year-old female. The patient underwent surgical debulking followed by 6000-rad irradiation. Due to a previous history of viral interstitial pneumonitis, the patient's diffusing capacity for carbon monoxide was only 39% and carmustine (BCNU) was believed to be contraindicated. The patient was therefore entered in a protocol with cisplatin at a dose of 60 mg/m^2 for 2 consecutive days every 4 weeks. She received five courses of cisplatin, with a decrease in tumor edema, decrease in midline shift, and tumor necrosis, as demonstrated by computerized tomographic scan. In view of data that suggest radiosensitization by platinum compounds (10,11), cisplatin was given on Days 1 and 28 of a 6-week course of radiation therapy.

In 5 months, the patient's tumor progressed. A second debulking procedure was performed 7 weeks subsequent to the previous dose of cisplatin. At surgery, necrotic tumor along with cystic changes were noted. Samples of both cystic fluid and tumor were removed by suction technique and assayed for platinum.

ANALYTIC METHODS

Tumor fragments were manually separated from blood and homogenized in five volumes of 0.9% NaCl. Only solid white or light pink tissue fragments were selected for homogenization. The homogenate was centrifuged at 105,000 × *g* for 60 minutes, and the supernatant (cytosol) was collected and saved. The pellet was resuspended in 0.9% NaCl and then diluted 1:1 with cold 20% trichloroacetic acid (TCA). The precipitate was sedimented by mild centrifugation. The 105,000 × *g* supernatant (cytosol) was subjected to centrifugal ultrafiltration utilizing CF-50 ultrafiltration membrane cones (Amicon Corp, Lexington, MA). The bloody matrix in which the tumor

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[1] Received Nov 2, 1981; revised Apr 27, 1982; accepted May 10, 1982.

[2] Clinical Fellow of the American Cancer Society.

[3] Vincent T. Lombardi Cancer Center, Georgetown University School of Medicine, Washington, DC.

[4] Laboratory of Medicinal Chemistry and Biology, Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD.

[*] *Reprint requests to:* Eric M. Bonnem, MD, Division of Hematology-Oncology, George Washington University, 2150 Pennsylvania Ave, NW, Washington, DC 20037.

fragments were originally found was centrifuged for 1 minute in an Eppindorf model 5412 centrifuge to sediment any tissue fragment or cells. The clear but red supernatant was then analyzed. Cystic fluid was analyzed without further treatment. All samples (tumor fragments, cystic fluid, and blood) were analyzed for protein content by the method of Lowry et al (12) and for platinum content by atomic absorption spectrophotometry as previously described (2). The limit of detection of the analytic method for platinum is 10 ng/ml.

RESULTS

The content of platinum in various tumor fractions and in the two fluids is presented in table 1. Of importance is the low but easily detectable concentration of platinum in the tumor several weeks after the last dose of cisplatin. Also of interest is the lack of detectable platinum present in ultrafiltrates and the TCA supernatant, indicating that platinum present in the tumor and fluids is bound to high-molecular-weight molecules.

DISCUSSION

Malonato 1,2-diaminocyclohexaneplatinum-II has been the only analog of platinum detected at measurable levels in the implantable rat tumor model (9). Other analogs of platinum, including cisplatin, have been recovered in brains of experimental animals at various times after treatment (1,2,5–8). Concentrations were always low and were usually near zero by the end of the first week after treatment. Interestingly, Hoeschele and vanCamp (5) have reported an increase in brain retention of platinum in tumor-bearing relative to control animals treated with cisplatin. Lown et al (6) showed a redistribution of platinum into brains beginning 3–7 days after treatment of mice with platinum sulfate [$Pt(SO_4)_2$].

There are laboratory data to suggest that cisplatin increases radiation sensitivity (11). *cis*-Dichlorobis-(cyclopentylamine)platinum (II), an analog of cisplatin, has been proposed as a radiation sensitizer in the Chinese hamster ovary model, causing such cells to accumulate in the radiosensitive G_1 phase (13). Increasing numbers of radiosensitive G_1 cells may explain the enhanced response following cisplatin and radiation combinations (14). In addition, cisplatin given with radiation has been shown to reduce the growth rate of three solid mouse tumors when compared to radiation alone (15). It has also been demonstrated both in vitro and in vivo that therapeutic potentiation occurs if a mouse mammary carcinoma is treated with cisplatin prior to irradiation (16). Thus, because of the potential for hypoxic cell sensitization, cisplatin was given to this patient during radiation therapy.

The standard treatment of glioblastoma multiforme has been surgery followed by radiation and BCNU therapy. The present results show that when cisplatin is administered, it can cross the blood-brain barrier and enter brain tumor tissue; there, it is apparently tightly bound. Easily measurable quantities of platinum were still present in our patient 7 weeks after the last dose of cisplatin. It would appear that a malignant neoplasm of the brain is capable of violating the blood-brain barrier, allowing penetration of cisplatin into the malignant tissue.

TABLE 1.—Platinum content of tumor fractions and brain fluids

Tissue	Platinum	
	µg/g or ml	ng/mg of protein
Tumor homogenate	1.60	7.8
105,000 × *g* supernatant*	0.09	3.8
105,000 × *g* pellet†	1.54	22.9
Cystic fluid*	0.09	3.4
Blood*	0.16	—

*Ultrafiltrate had no detectable platinum.
†TCA-soluble supernatant had no detectable platinum.

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CLINICAL TRIALS SUMMARIES

Phase II Study of DON in Patients With Previously Treated Advanced Lung Cancer[1]

Robert T. Eagan,* Stephen Frytak, William C. Nichols, Edward T. Creagan, and James N. Ingle[2]

DON, an antitumor antibiotic isolated from cultures of *Streptomyces* and a structural analog of L-glutamine, functions as a glutamine antagonist (1). In an earlier phase II study of DON given daily, either orally or iv, two of nine patients with lung cancer showed apparent tumor regression (2). Because of significant mucositis and diarrhea from that schedule, DON had limited evaluation. Recently, we completed a phase I evaluation of an intermittent 3-day iv regimen (3) in which nausea and vomiting were the dose-limiting toxic effects. A dose of 160 mg/m^2 iv daily for 3 consecutive days every 3 weeks was recommended for phase II studies.

The pretreatment characteristics, drug dose and schedule of DON, survival, toxicity, and response rate are shown in table 1. Of the 21 patients with prior chemotherapy, 14 had had prior tumor regressions (eight of 15 patients with non-small cell carcinoma and six of six with small cell carcinoma). The primary lung tumor was the major indicator disease in 20 of 22 patients. Fifteen patients completed one course of treatment and were observed to have tumor progression by the first re-evaluation in 3 weeks. Three patients had less than one complete course of DON (refused further treatment because of gastrointestinal toxicity) and had no regressions. Four patients had brief periods of stability (3–12 weeks) prior to disease progression. Acute nausea, vomiting, and diarrhea were common and significant and were not well-controlled or prevented by prochlorperazine or prophylactic diphenoxylate hydrochloride with atropine sulfate. Occasional mild to moderate nausea, vomiting, diarrhea, constipation, and stomatitis were reported in the 3-week interval following DON. Interval myelosuppression was noted in 13 of the 19 evaluable patients (68%).

TABLE 1.—Characteristics, survival, toxic reactions, and response rate of patients with lung cancer treated with DON*

Characteristic	No. of patients (No. with prior chemotherapy)
Total entered	23
Total eligible and evaluable†	22 (21)
Sex	
Male	17
Female	5
Age (yrs)	
Median: 61	
Range: 46–71	
Performance status‡	
1	8
2	8
3	6
Tumor	
Adenocarcinoma	9 (9)
Squamous cell carcinoma	4 (3)
Small cell carcinoma	6 (6)
Large cell carcinoma	3 (3)
Regressions§	0
Survival (days) ¶	
Median: 107	
Range: 19–365 +	
Toxic effects	
Nausea and vomiting	
Moderate: 57%	
Severe: 30%	
Diarrhea	
Mild to moderate: 30%	
Severe: 7%	
Hematologic nadirs	
Wbc count < 4000/mm^3**	9/19
Platelet count < 130,000/mm^3 ††	9/19

*160 mg/m^2 given as 10–15-min iv infusion in 50 ml of 0.9% saline, daily for 3 consecutive days every 3 weeks.

†1 patient ineligible because of gastric cancer at autopsy.

‡Eastern Cooperative Oncology Group criteria.

§Primary indications of disease: lung (20 patients), liver (1), and nodes (1).

¶Median survival in days (range) by tumor type: adenocarcinoma—71 (19–196); squamous cell carcinoma—155 (139-212 +); small cell carcinoma—63 (23–115); and large cell carcinoma—121 (101–365 +).

**Median wbc count nadir (range): 3000 (2100-3700); median Day (range): 12 (6–21).

††Median platelet count nadir (range): 69,000 (31,000–120,000); median Day (range): 12 (8–14).

DISCUSSION

No tumor regressions were noted among 22 patients with advanced lung cancer and one patient with advanced gastric cancer. All but one of the patients had received extensive prior chemotherapy. Two of 11 patients treated subsequent to disease progression during DON therapy had tumor regression. Nausea and vomiting were fre-

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[1] Received Jan 18, 1982; revised Apr 22, 1982; accepted May 6, 1982.

[2] Division of Medical Oncology, Mayo Clinic, Rochester, MN.

Reprint requests to: Robert T. Eagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

quent and significant. Myelosuppression occurred in 68% of the evaluable courses of DON. DON, given iv on a 3-day intermittent schedule, showed significant gastrointestinal toxicity and appears to have little activity in patients with heavily pretreated lung cancers.

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Phase II Trial of Spirogermanium in Breast Adenocarcinoma: A Cancer and Leukemia Group B Study[1]

Daniel R. Budman,* Sandra Ginsberg, Michael Perry, Vivian Weinberg, Philip Schein, John Hanson, Morton Coleman, and William Wood[2]

The results of previous studies (1,2) suggest that spirogermanium, a germanium compound with cytotoxic activity, has antitumor activity in breast (1) and ovarian carcinoma (3). Phase I trials had suggested a lack of myelotoxicity (1,2,4), making this agent attractive for phase II trials.

Female patients with histologically documented breast adenocarcinoma who were not candidates for surgical resection, radiation therapy, or standard chemotherapy were eligible for the study. No patient had received more than two chemotherapeutic regimens.

Cancer and Leukemia Group B (CALGB) criteria included: (*a*) measurable disease; (*b*) ambulatory performance status (PS) ≤ 3; (*c*) total wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, serum bilirubin ≤ 1.5 mg/dl, and serum creatinine ≤ 1.5 mg/dl; (*d*) no prior therapy for 4 weeks before entry; (*e*) anticipated survival > 2 months; and (*f*) informed consent.

Patients were treated with a multidose schedule of iv infusion for 1 hour at a dose of 80 mg/m^2 (4), receiving spirogermanium on Monday, Wednesday, and Friday for 2 weeks, then twice a week, on Tuesday and Thursday, for 2 weeks. After ten treatments, patients received a single weekly infusion until disease progression occurred.

Patients were evaluated weekly for response and toxic effects, using standard criteria (5); serum electrolytes, glucose, BUN, creatinine, bilirubin, alkaline phosphatase, SGOT, and SGPT were measured every 2 weeks.

Thirty-six patients were entered in the study (table 1); four did not meet eligibility requirements and two did not receive treatment according to the specified schedule. Twenty-three patients completed a minimum of 3 weeks of therapy, with six patients receiving > 9 weeks of treatment. Death within 3 weeks of start of protocol was scored as progressive disease.

Toxic effects were noted in 50% of these patients, paralleling preclinical animal toxicology and previous phase I studies (1,2,4). Minor myelosuppression occurred in 17% of the patients (table 1). Neurologic toxicity, prominent in preclinical and phase I studies, was not dose-limiting in most patients. One patient had grand mal seizures, which may have been drug-related.

No major responses were seen among the 30 patients evaluated, indicating that spirogermanium did not have at least a 15% frequency of response at the 99% confidence level in this population.

TABLE 1.—Patient characteristics and treatment results

Characteristic	
No. of patients entered/evaluable	36/30
Age at entry (yrs)	
Mean	58.4
Range	42–78
% of patients with initial CALGB PS	
0	20
1	47
2	30
3	3
No. of dominant disease sites	
Visceral	67
Bone*	10
Soft tissue	23
% of patients with prior chemotherapy	
1 combination	45
2 combinations	55
Prior anthracycline	79
Duration of treatment (wks)	
Mean: 5	
Range: 1–9	
No. of patients with—	
CR/PR†	0
Stable disease	3
Disease progression	27
% of patients with—	
Leukopenia ($\leq$ 3000 cells/μl)	17
Thrombocytopenia ($\leq$ 100,000 cells/μl)	4
Congestive heart failure	4
Neurologic effects	11‡
Nausea and vomiting	17
Phlebitis	7

*Scintiscan did not show measurable disease, although it could indicate dominant disease.

†CR—complete response; PR—partial response.

‡Includes patient who had fatal grand mal seizure.

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[1] Received Jan 18, 1982; revised May 6, 1982; accepted May 17, 1982.

[2] Cancer and Leukemia Group B, Brookline, MA.

**Reprint requests to:* Daniel R. Budman, MD, Division of Medical Oncology, Department of Medicine, North Shore University Hospital, 300 Community Drive, Manhasset, NY 11030.

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CLINICAL TRIALS SUMMARIES

Phase II Trial of Etoposide in the Management of Advanced or Recurrent Endometrial Carcinoma: A Gynecologic Oncology Group Study[1,2]

R. E. Slayton,[3] J. A. Blessing,[4] and G. Delgado[5,*]

Etoposide is a semisynthetic podophyllotoxin derivative. In contrast to other podophyllotoxins that are spindle poisons, etoposide appears to block entry of cells into mitosis, acting during the late S and G_2 phases of the cell cycle (1,2).

Responses have been observed in patients with lymphoma (3,4), leukemia (4,5), brain tumor (6), lung cancer (7), breast cancer (8), bladder cancer (9), etc. Toxic effects include leukopenia, thrombocytopenia, alopecia, nausea, vomiting, and diarrhea, all of which are generally reversible when therapy is discontinued. Headache, fever, and hypotension have been recorded infrequently. Recently, etoposide has been employed in combination for patients with lung cancer (10) and lymphoma (11,12).

In 1979, we reported results of phase II trials with etoposide conducted by the Gynecologic Oncology Group (GOG) in the treatment of advanced epithelial tumors of the ovary and squamous cell carcinoma of the cervix (13); two partial responses were observed in the former group (24 patients), while no responses were noted in the latter group (31 patients). A similar phase II GOG study was conducted to investigate the role of etoposide in the management of advanced or recurrent endometrial carcinoma.

Etoposide (100 mg/m^2 diluted in 150 ml of normal saline) was administered iv over a 45-minute period on Days 1, 3, and 5. The cycle was repeated every 28 days. A complete description of materials and methods is contained in an earlier paper (13).

Complete response is defined as the disappearance of all gross evidence of disease for at least 1 month. Partial response is defined as a ≥ 50% reduction in the product of the largest diameter and its perpendicular of each measurable lesion for a period of at least 1 month. Increasing disease is defined as a ≥ 50% increase in the product of the largest diameter and its perpendicular of each measurable lesion documented on two separate examinations at least 2 weeks apart or the appearance of any new lesion within 3 months of entry in the study. Stable disease is defined as a response not meeting the above criteria.

Patient characteristics, response to therapy, and adverse effects are summarized in table 1. Among 29 patients evaluated, only one partial response of 2 months' duration was noted; 13 patients had stable disease, while 15 had increasing disease. Leukopenia was the most serious adverse effect and was observed in 16 patients, with a median wbc count nadir of 2350/mm^3 (range, 250–3700). Thrombocytopenia was reported in 11 patients, with a median platelet count nadir of 94,000/mm^3 (range, 19,600–144,000).

We conclude that etoposide at the dose and schedule tested has negligible activity in patients with advanced endometrial carcinoma previously treated with combination chemotherapy.

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[1] Received Feb 22, 1982; revised May 6, 1982; accepted May 19, 1982.

[2] This study was conducted by the Gynecologic Oncology Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in this study: University of North Carolina School of Medicine, Chapel Hill (CA-23073); Georgetown University Hospital, Washington, DC (CA-16938); Bowman-Gray School of Medicine, Wake Forest University, Winston Salem, NC (CA-21946); Hahnemann Medical College/Jefferson Medical College, Philadelphia, PA (CA-12478); The Milton S. Hershey School of Medicine, Pennsylvania State University, Hershey (CA-16386); University of California Medical Center, Irvine (CA-23765); Indiana University Medical Center, Indianapolis (CA-21720); University of Nebraska Medical Center, Omaha (CA-23084); University of Oregon Health Sciences Center, Portland (unfunded); Duke University Medical Center, Durham, NC (CA-12534); University of Rochester Medical Center, NY (CA-12482); State University of New York, Buffalo (CA-12476); The Albany Medical College of Union University, NY (unfunded); Tufts New England Medical Center, Boston, MA (unfunded); Illinois Cancer Council, Chicago (CA-27806); University of California Medical Center at Los Angeles (CA-13630); and Albert Einstein College of Medicine, New York, NY (unfunded).

[3] Department of Medicine, Section of Medical Oncology, Rush-Presbyterian-St Luke's Medical Center, Chicago, IL.

[4] Roswell Park Memorial Institute, Buffalo, NY.

[5] Georgetown University, Washington, DC.

[*] *Reprint requests to*: Gynecologic Oncology Group Headquarters, 1234 Market St, Suite 430, Philadelphia, PA 19107.

TABLE 1.—Phase II study of etoposide in advanced endometrial carcinoma

Age (yrs)	Original stage	Previous treatment*	Mos† to etoposide	GOG performance status	Response to etoposide‡	Courses of etoposide	Toxic effects
42	IV	ADR–CTX–5-FU–Megace	16	1	ID	1	Leukopenia (2900 cells/mm^3)
44	II	RT, ADR–CTX–5-FU–Megace	22	3	SD	3	Nausea, weakness
48	IV	Provera–ADR–CTX	5	0	ID	2	—
49	IV	MELPH–5-FU–Megace	3	2	ID	1	—
53	I	RT, MELPH–5-FU–Megace, CIS	60	1	SD	12	Minimal hair loss, fatigue
54	II	RT, MELPH–5-FU–Megace, CIS	66	0	SD	7	Leukopenia (3700 cells/mm^3), nausea
54	II	RT, MELPH–5-FU–Megace	15	2	SD	2	Leukopenia (250 cells/mm^3), thrombocytopenia (19,600 cells/mm^3)
56	I	RT, ADR–CTX–5-FU–Megace	17	2	ID	1	—
57	II	RT, ADR–CTX–Provera	6	2	ID	1	—
61	I	RT, ADR, PIP	45	2	SD	2	Leukopenia (1150 cells/mm^3), thrombocytopenia (88,000 cells/mm^3), weakness, fever
62	III	ADR–CTX–5-FU–Megace	15	2	SD	4	Leukopenia (3500 cells/mm^3), thrombocytopenia (110,000 cells/mm^3), nausea, vomiting
63	IV	ADR–CTX–5-FU–Megace, CIS	11	1	ID	2	Leukopenia (2400 cells/mm^3), thrombocytopenia (107,000 cells/mm^3), alopecia
63	—	RT, Provera–Megace, ADR–CTX, CTX–Megace	124	1	SD	10	Leukopenia (2300 cells/mm^3), alopecia
63	—	RT, ADR–CTX–5-FU–Megace, PIP	51	2	ID	1	Thrombocytopenia (67,000 cells/mm^3)
64	III	RT, ADR–Megace	37	1	ID	2	—
64	IV	MELPH–5-FU–Megace	6	1	ID	1	Nausea
64	III	RT, ADR–CTX–5-FU–Megace	10	2	SD	3	Leukopenia (3200 cells/mm^3)
65	III	RT, Megace, ADR–CTX	10	1	ID	1	—
66	I	RT, MELPH–5-FU–Megace	22	2	SD	10	Fatigue, alopecia
67	I	RT, ADR–CTX–5-FU–Megace	28	1	SD	2	Leukopenia (1800 cells/mm^3), thrombocytopenia (144,000 cells/mm^3)
68	III	ADR–CTX–5-FU–Megace	11	2	PR	13	—
69	III	RT, Megace, ADR	11	2	ID	2	Leukopenia (800 cells/mm^3), thrombocytopenia (55,000 cells/mm^3), skin breakdown
69	III	Megace, Provera, ADR–CTX–5-FU–Delalutin, RT	25	2	ID	1	Leukopenia (1100 cells/mm^3)
70	I	MELPH–5-FU–Megace, RT	8	3	ID	2	Leukopenia (2500 cells/mm^3), thrombocytopenia (25,000 cells/mm^3)
73	I	RT, MELPH–5-FU–Megace	56	2	SD	8	Leukopenia (900 cells/mm^3), thrombocytopenia (104,000 cells/mm^3), anemia
74	I	RT, Provera	43	1	SD	5	Leukopenia (3168 cells/mm^3)
77	IV	MELPH–5-FU–Megace, ADR	12	0	ID	3	Thrombocytopenia (144,000 cells/mm^3), nausea

77	—	RT, MELPH–5-FU–Megace	55	3	ID	2	Leukopenia (1700 cells/mm³), nausea, vomiting
78	III	RT, MELPH–5-FU–Megace	10	1	SD	3	Leukopenia (3500 cells/mm³), thrombocytopenia (94,000 cells/mm³), nausea, vomiting

*ADR = doxorubicin; CTX = cyclophosphamide; RT = radiation therapy; MELPH = melphalan; CIS = cisplatin; PIP = piperazinedione.

†From diagnosis.

‡ID = increasing disease; SD = stable disease; PR = partial response.

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CLINICAL TRIALS SUMMARIES

Gallium Nitrate in Advanced Soft Tissue and Bone Sarcomas: A Southwest Oncology Group Study[1,2]

John H. Saiki,[3] Laurence H. Baker,[4] Ronald L. Stephens,[5] Carol J. Fabian,[5] Eric H. Kraut,[6] and William S. Fletcher[7,*]

Gallium nitrate, an anhydrous salt of gallium (a naturally occurring heavy metal), was reported in 1975 to have significant antitumor activity in animal systems (1). Two clinical phase I trials reporting activity in bone and soft tissue sarcomas led to the study of gallium nitrate in advanced soft tissue and bone sarcomas by the Southwest Oncology Group (2,3).

PATIENTS AND METHODS

Thirty-two patients with advanced, histologically confirmed soft tissue and bone sarcomas were entered in the study (table 1). Entrance requirements included written informed consent in accordance with institutional and FDA guidelines, life expectancy of at least 6 weeks, and normal hematopoietic, hepatic, and renal functions.

All patients were hydrated with iv fluids followed by the administration of gallium nitrate at a dose of 700 mg/m^2 as a 30-minute iv infusion. Treatments were administered at 2-week intervals. An adequate trial was defined as two courses of therapy (4 weeks).

RESULTS

Response

No complete or partial responses were observed. Gallium scans were performed in seven patients. In all seven patients, activity was noted in regions of the primary and/or metastatic site.

Toxicity

Leukopenia was mild (3–3.9 × 10^3 cells/mm^3) to moderate (2–2.9 × 10^3 cells/mm^3) in 30% of the evaluable patients. Mild nephrotoxicity (BUN 20–40 mg/100 ml; creatinine 1.2–2 mg/100 ml) occurred in 30% of the patients but was severe (BUN > 100 mg/100 ml; creatinine > 4 mg/100 ml) in only one. Nauseas and vomiting were mild to moderate in slightly more than one half of evaluable patients. Mild hearing impairment developed in one patient. Neuropathy was not observed.

TABLE 1.—Patient characteristics, histologic subgroup, and response

Characteristic	No. of patients
Total evaluable	31
Sex	
Female	12
Male	19
Age in yrs	
Median: 52	
Range: 15–78	
Histologic subgroup	
Soft tissue sarcoma	24
Bone sarcoma	7
Prior therapy	
Doxorubicin combinations	26
Other combinations	3
None	2
Radiotherapy	18
Response to gallium nitrate (700 mg/m^2)	0
Toxic effects	
Leukopenia*	10
Nephrotoxicity	
Mild*	7
Severe‡	1
Nausea and vomiting (mild to moderate)	16
Hearing impairment (mild)	1

*2–3.9 × 10^3 cells/mm^3.
†BUN 20–40 mg/100 ml; creatinine 1.2–2 mg/100 ml.
‡BUN > 100 mg/100 ml; creatinine > 4 mg/100 ml.

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[1] Received Feb 25, 1982; revised Apr 28, 1982; accepted May 19, 1982.

[2] Supported in part by Public Health Service grants CA-12213, CA-12028, CA-12644, CA-04920, CA-21116, CA-26766, CA-04919, CA-03389, CA-22411, CA-27057, CA-16385, CA-16957, CA-20319, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] University of New Mexico Medical Center, Albuquerque.

[4] Wayne State University School of Medicine, Detroit, MI.

[5] University of Kansas Medical Center, Kansas City.

[6] Ohio State University Hospital, Columbus.

[7] University of Oregon Health Sciences Center, Portland.

[*] *Reprint requests to:* Southwest Oncology Group (SWOG-7911), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

DISCUSSION

This phase II study of gallium nitrate failed to demonstrate activity in patients with soft tissue and bone sarcomas. In prior clinical phase I trials (2–4), one of three patients with osteogenic sarcoma (2) and one of 17 with soft tissue sarcoma (3) showed a partial response. We conclude that, at the dose and schedule used in this study, gallium nitrate does not appear to have activity in previously treated patients with advanced soft tissue or bone sarcomas.

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CLINICAL TRIALS SUMMARIES

Phase II Trial of Spirogermanium in Advanced Epithelial Carcinoma of the Ovary[1]

Lora Weiselberg,[2,*] Daniel R. Budman,[2] Philip Schulman,[2] Vincent Vinciguerra,[2] Thomas J. Degnan,[2] and Mark Pasmantier[3]

Spirogermanium is an azaspiran-germanium compound that has had no hematologic, hepatic, or renal toxicity in phase I studies (1). Initial studies suggested antitumor activity in pretreated patients with ovarian carcinoma (2), thus prompting a phase II trial in patients with recurrent ovarian carcinoma.

In addition to giving informed consent, patients eligible for the study had to have (*a*) histologically documented inoperable or recurrent epithelial carcinoma of the ovary, (*b*) failure of a trial of standard therapy defined as an alkylating agent plus cisplatin in patients with a creatinine clearance > 60 ml/min, (*c*) Cancer and Acute Leu-

TABLE 1.—Patient characteristics and treatment results

Age (yrs)	CALGB PS	Prior treatments*	Response to spirogermanium	No. of doses of spirogermanium	Toxic effects
42	2	ALK, CTX, HMM, MTX, 5-FU, CDDP, AMSA	Disease progression	11	None
46	2	CTX, MTX, 5-FU, ADR, CDDP, HMM	Disease progression	14	None
53	2	CTX, MTX, ADR, 5-FU, HMM, CDDP	Disease progression	11	None
56	2	CTX, MTX, ADR, HMM, CDDP, 5-FU, AMSA, VINCR, BLEO	Inevaluable	3	Blurred vision
49	3	CTX, ADR, CDDP, HMM	Disease progression	11	None
49	2	CTX, ADR, CDDP, HMM	Disease progression	12	Light-headedness
49	0	CTX, CDDP, HMM	Disease progression	16	Light-headedness
44	3	CTX, ADR, CDDP, HMM, VLB	Disease progression	10	Psychosis
67	2	CTX, ADR, CDDP, HMM, MMC, MTX	Disease progression	10	None
65	2	CTX, ADR, CDDP, HMM, TAMOX, VLB	Disease progression	18	Confusion, light-headedness, agitation
72	2	CTX, ADR	Stabilization	12	Leukopenia
57	2	CTX, ADR, CDDP, HMM, 5-FU, LEUK	Disease progression	11	Light-headedness
60	1	CTX, ADR, CDDP, HMM, VLB	Disease progression	15	None
62	1	LEUK, CTX, ADR, CDDP, HMM	Disease progression	10	Light-headedness

*ALK = melphalan, CTX = cyclophosphamide, HMM = hexamethylmelamine, MTX = methotrexate, CDDP = cisplatin, VINCR = vincristine, BLEO = bleomycin, VLB = vinblastine, LEUK = chlorambucil, TAMOX = tamoxifen, MMC = mitomycin, ADR = doxorubicin.

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[1] Received Apr 28, 1982; accepted May 11, 1982.

[2] Don Monti Division of Oncology, Department of Medicine, North Shore University Hospital, Manhasset, NY, and Department of Medicine, Cornell University Medical College, New York, NY.

[3] Division of Medical Oncology, Department of Medicine, The New York Hospital, Cornell University Medical College, New York, NY.

[*] *Reprint requests to:* Lora Weiselberg, MD, Don Monti Division of Oncology, Department of Medicine, North Shore University Hospital, 300 Community Dr, Manhasset, NY 11030.

kemia Group B (CALGB) performance status (PS) ≤ 3 (ambulatory), (*d*) measurable disease, (*e*) expected survival > 2 months, and (*f*) total bilirubin < 1.5 mg/dl and creatinine clearance > 30 ml/min.

All patients were treated by 60-minute infusion of spirogermanium on the following schedule: three times weekly during Weeks 1 and 2, twice weekly during Weeks 3 and 4, and once weekly at Week 5 and thereafter. The initial dose level was 60 mg/m^2; in the absence of toxic effects this was escalated to 80 mg/m^2 at Week 2 (3). All patients were evaluated for toxic reactions and response on a biweekly basis. Responses were evaluated according to previously defined criteria (2).

Of the 14 patients entered in the study, all were evaluable for toxic reactions and 13 were evaluable for response.

Significant neurotoxic reactions were observed in one half of the patients treated in this group (table 1), despite administration of spirogermanium by slow iv infusion and gradual dose escalation. A previous phase I trial (3) also demonstrated lower tolerance to spirogermanium in patients with a poor PS. One patient exhibited a mild leukopenia of 3500 cells/mm^3 at Day 8, which rose to 4600 cells/mm^3 on Day 17 despite continuation of spirogermanium at full dose.

No major responses were seen in 13 consecutive evaluable patients, indicating that the therapeutic antitumor effect in previously treated patients with ovarian carcinoma is < 20%, within 90% confidence limits. This study could not confirm activity of spirogermanium in previously treated patients with ovarian carcinoma.

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LETTERS

Lack of Correlation Between Effects of Anthracyclines on In Vitro Cardiac Guanyl Cyclase and In Vivo Cardiotoxicity[1]

Lehotay et al (1) recently reported that anthracyclines and anthracenediones inhibited, and occasionally stimulated, rat heart guanylate cyclase in vitro. Based on the observation that daunorubicin and doxorubicin were more potent inhibitors than the less cardiotoxic compounds aclacinomycin A and 7-OMEN, the authors speculated that a quantitative or causal relationship may exist between cardiotoxicity and inhibitory activity of cardiac guanylate cyclase. Since we have tested most of the compounds in a rat cardiotoxicity model, it was of interest to determine whether or not the in vivo data fit this hypothesis.

The effects of 26 derivatives on cardiac guanylate cyclase activity are presented in table 1 together with the minimal cumulative cardiotoxic dose (MCCD) as determined in the rat model. In this experiment drugs were injected ip five times per week at two or more doses. ECGs were recorded, and the total dose causing a significant prolongation of the QRS interval was determined (2).

There was no significant correlation between in vitro enzyme inhibition and ECG effects in vivo. This is not surprising, since the pharmacokinetic complexities are not considered in the in vitro system. A case in point is the *N,N*-dimethyl analog of doxorubicin, which is a weak inhibitor of guanylate cyclase. In the intact rat it caused greater ECG changes and more serious structural damage of the heart muscle than doxorubicin. The explanation was an eightfold higher accumulation of the *N,N*-dimethyl analog in the rat hearts after 1 week of treatment (3).

The ECG changes induced in rats by doxorubicin and most of its derivatives develop gradually and, if the dose is properly adjusted, in the absence of serious general toxic effects. But with each new injection the QRS interval becomes longer. However, some compounds elicit a different response. For example, carubicin did not induce ECG changes until the rats began to die. The electron microscopic evaluation of the heart muscle also disclosed qualitative differences: instead of prominent dilatation of the sarcoplasmic reticulum, which is typical for anthracyclines, carubicin caused mainly atrophy of the myofibrils (4). Another type of response was observed with AD-32: ECG changes of the same type as seen with doxorubicin developed promptly (MCCD, 48 mg/kg), but with continued treatment almost no further deterioration of the ECG occurred. From this discussion it is evident that biologically relevant differences exist between closely related anthracyclines. These are, of course, not recognized if only one biologic quality is measured.

The hydrazone derivatives tested by Lehotay et al (1) did not inhibit, but stimulated, guanylate cyclase activity. In the rat in vivo cardiotoxicity model, these compounds had relatively weak cardiotoxic properties. Moreover, zorubicin, the most widely studied hydrazone derivative, differed from doxorubicin also with regard to its effect on heart mitochondrial function (5).

It is encouraging to note that it is now possible to measure a variety of biologic effects of anthracycline antibiotics on the heart. Although the relevance of these effects for the development of cardiomyopathy is not well-understood, it is hoped that the better ability to characterize the biologic spectrum of new anthracyclines will help in the discovery of new derivatives with more favorable margins of safety.

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Gerhard Zbinden

Institute of Toxicology
Swiss Federal Institute of Technology
and University of Zurich
Schwerzenbach, Switzerland

March 25, 1982

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[1] Supported by a grant from the Swiss National Science Foundation. The compounds were provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, under contract 236-77-C-0410 CC.

TABLE 1.—Effects of test compounds on cardiac guanylate cyclase and on rat ECGs in vivo

NSC No.	Name	% change in guanylate cyclase from control*	MCCD (mg/ kg)
	Parent compounds and short-chain derivatives		
256942	4-Epi-doxorubicin	–56	16
123127	Doxorubicin	–44	11
143491	Doxorubicin 3-oxime	–24	48
263673	Daunorubicin	–23	12
180024	Carubicin	–22	*See* text
256439	Demethoxydaunorubicin	–22	3.5
149684	Doxorubicin-14-nicotinate	–21	20
268708	Daunorubicin-9-desacetyl	–13	33
	Anthracenedione derivatives		
279836	Dihydroxyanthracenedione	–24	8
196473	9, 10-Anthracenedione	–20	128
	Sugar or long-chain hydrocarbon derivatives		
246071	Doxorubicin, *N*-dodecanoyl	–8	72
261057	Doxorubicin, 14-stearoyl glycolate	–4	40
261045	Doxorubicin, *N*,*N*-dimethyl	–2	6 (*see* text)
269148	7-OMEN	+2	45
246131	AD-32	+3	48 (*see* text)
258812	Daunorubicin, *N*,*N*-dimethyl	+5	11
267703	Quelamycin	+6	11.5†
268242	Daunorubicin, *N*,*N*-dibenzoyl	+14	125
208734	Aclacinomycin A	+19	28
	Hydrazone derivatives		
146496	Daunorubicin gycoloylhydrazone HCl	+2	40
221265	Daunorubicin, *m*-nitrobenzoylhydrazone	+9	32
237672	Daunorubicin, 3,4-dichlorobenzoylhydrazone	+19	20
164011	Zorubicin	+25	24
219977	Daunorubicin, *p*-chlorobenzoylhydrazone	+26	48
233853	Doxorubicin, octanoylhydrazone	+26	40
266210	Daunorubicin, bis-succinoylhydrazone	+36	60

*Data from ref 1.

†Calculated as doxorubicin equivalents. Spearman rank correlation coefficent = 0.32 ($P > 0.1$).

Acute ECG Changes Associated With AMSA Treatment

AMSA is a relatively new acridine derivative with antitumor activity. Previous phase I and II clinical trials using this agent have revealed that myelosuppression is the major toxic side effect (1). Other side effects include nausea and vomiting, stomatitis, alopecia, local phlebitis, allergic dermatitis, hepatotoxicity, and fatigue (2–4). Acute cardiac toxicity has been reported to occur during or shortly after the administration of the drug. This toxic effect may be related to concomitant hypokalemia (5).

In two patients who were being treated with AMSA, we observed acute ECG changes that appeared to be transient and not related to electrolyte abnormalities. More importantly, these laboratory findings were not related to any clinical signs or symptoms of cardiac toxicity.

CASE REPORTS

Patient 1

A 63-year-old white male with bladder cancer metastatic to the lungs had had disease progression during prior therapy including a combination of cyclophosphamide, doxorubicin, and cisplatin. He began AMSA therapy according to a Southwest Oncology Group protocol at a dose of 90 mg/m^2 by iv infusion. No abnormalities were noted in the ECG after the first dose of 170 mg. Following the second dose, the ECGs showed a significant change. While the pretreatment study (fig 1A) was essentially normal, an ECG taken 1 hour after AMSA infusion (fig 1B) revealed ST-segment alterations and T-wave inversion consistent with anterolateral ischemia. A study 24 hours later (fig 1C) showed that the ECG had

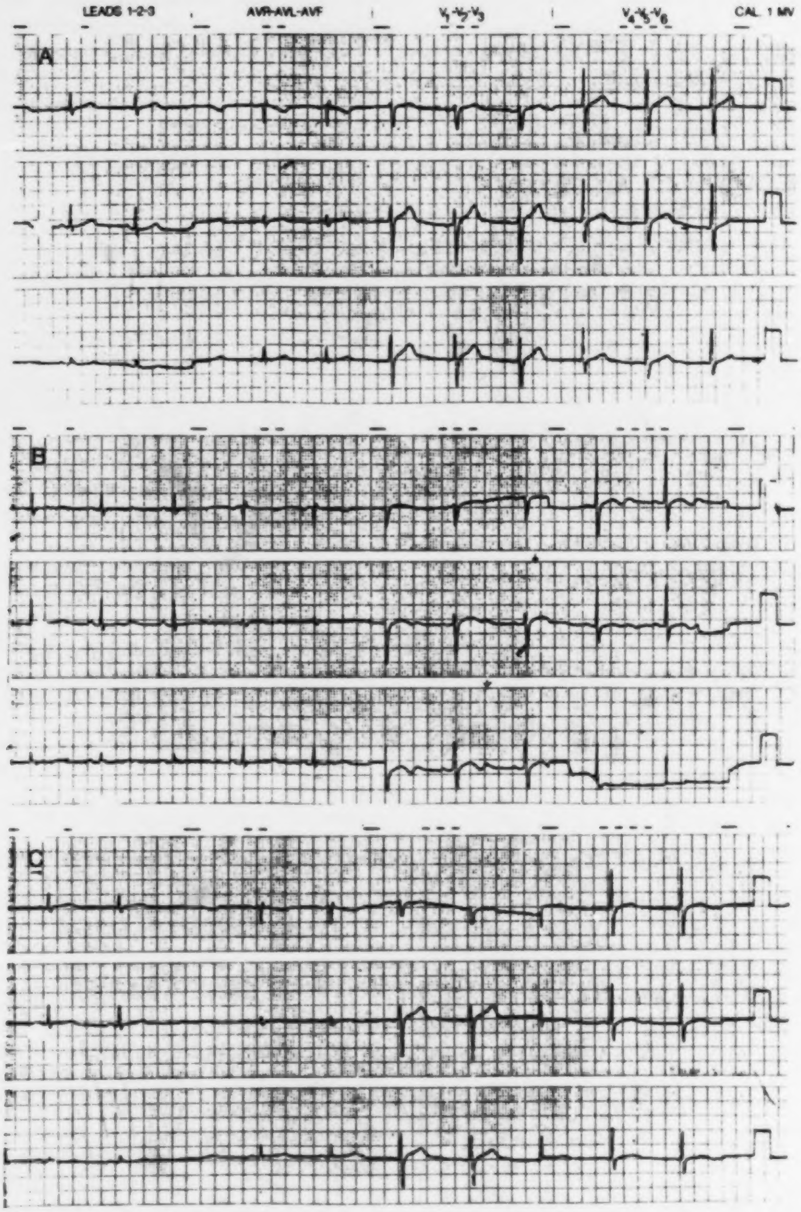
LEADS 1-2-3
AVR-AVL-AVF
V1-V2-V3
V4-V5-V6
CAL. 1 MV
A
B
C

FIGURE 1.—ECGs for patient 1: prior to AMSA infusion (A), 1 hr after AMSA infusion (B), and 24 hrs after AMSA infusion (C).

reverted once again to the normal pretreatment pattern. The patient was totally asymptomatic during the drug infusion and remained so during the entire treatment period; pretreatment serum potassium was 3.6 mEq/liter.

Prior to his next dose of AMSA, the patient was admitted to the hospital and underwent extensive cardiac evaluation. His pretreatment and posttreatment ejection fractions, cardiac enzymes, electrolytes, and pyrophosphate scans were all within normal limits. Pretreatment thallium scan was abnormal but showed no change following therapy. ECGs once again showed changes consistent with anterolateral ischemia 1 hour after the patient received AMSA. The ECG again returned to normal 12–24 hours after the infusion. Cardiac monitoring was performed for 24 hours and no arrhythmias were observed. The patient remained asymptomatic during and after the treatment. He received a fourth course of AMSA; ECG showed transient ischemic changes, but there were no clinical symptoms. The total AMSA dose was 680 mg. The patient did have the expected myelosuppression following each course but had only minimal nausea. No other toxic effect was noted.

Patient 2

A patient with metastatic prostate cancer treated with AMSA in a Southwest Oncology Group protocol had similar transient ECG changes following his second course of the drug. Complete cardiac evaluation failed to reveal any additional evidence of cardiac toxicity. This patient failed treatment after a total of three courses of AMSA. He remains without any clinical evidence of cardiac abnormalities > 6 months after receiving AMSA.

DISCUSSION

Because of reports of cardiac arrhythmias occurring with AMSA, we began doing frequent ECGs for patients receiving this treatment. The ST-T-wave changes described above were an unexpected finding. We were concerned that they might represent early signs of more severe cardiac toxicity. This fear was not substantiated by extensive cardiac evaluation. The transient nature of the ECG changes may represent a local irritant-like effect on the myocardium. It is unlikely that they represent any true ischemic changes in the heart, in view of normal pyrophosphate studies.

The relationship of our findings to the previously described arrhythmias is not clear. Our ECG abnormalities occurred in the presence of normal potassium and calcium levels and were not associated with rhythm disturbances. It is possible that the ischemic changes in ST-T-waves that we observed could be precursors of serious arrhythmias in the presence of hypokalemia or other metabolic abnormalities. We feel that patients receiving AMSA should continue to have pretreatment and posttreatment ECGs, electrolyte monitoring, and observation for arrhythmias. The presence of transient ECG alterations similar to those we observed should not of themselves be an indication to discontinue therapy with AMSA. Without associated clinical symptoms or evidence of any cardiac rhythm disturbance, treatment with AMSA should be continued in patients whose tumors appear to be responding to this new drug.

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Charles F. Miller

Hematology-Oncology Service
Tripler Army Medical Center
Honolulu, HI 96859

Niranjan Rajdev

Hematology-Oncology Section
Honolulu Medical Group
Honolulu, HI

March 29, 1982

Southwest Oncology Group Experience With Tamoxifen in Metastatic Melanoma

Although epidemiologic, biochemical, and clinical observations have suggested a role for endocrine factors in the growth and spread of malignant melanoma, prospective clinical trials of hormonal therapies in patients with metastatic disease have in general been disappointing. Occasional patients, however, appear to have significant responses to such agents.

Tamoxifen has been tested in metastatic melanoma by at least six investigators (1–6). Analyzed collectively, these studies have yielded a response rate of 10% (partial plus complete responses), lasting a median of 7 months. All of these studies used a fixed dose of tamoxifen ranging from 20 to 40 mg daily.

In 1978, the Southwest Oncology Group initiated a study of melanoma to determine the efficacy of tamoxifen given with a 7-day loading schedule at 40 mg/m^2 twice a day followed by a fixed dose of 10 mg/m^2 twice a day until disease progression. This schedule was selected in an attempt to overcome any reflex increase in estradiol production following initiation of tamoxifen. Such an increase theoretically may overcome the binding of tamoxifen to steroid receptors on tumor cells. Fabian et al (7) demonstrated that patients treated with this schedule achieved a steady-state blood level 3 weeks earlier than patients treated with the usual schedule of 10 mg orally twice a day.

Twenty-eight patients (20 males and eight females) with metastatic melanoma completed 1 month of prescribed therapy using this loading schedule and were evaluable. All patients had received prior chemotherapy and had measurable disease. Eleven patients (seven males and four females) had tumor biopsied for estrogen-receptor (ER) content; only one had an ER value > 3 fmols/mg.

Tamoxifen in this schedule was well-tolerated. One patient developed mild reversible thrombocytopenia (platelet count, 97,000/mm^3) and another had mild nausea. However, only one patient responded. This was a premenopausal female with ER-negative tumor, who had complete resolution of cutaneous and nodal metastases for 4$^1/_2$ months.

We conclude that this loading schedule does not increase the efficacy of tamoxifen in metastatic melanoma. We cannot share the enthusiasm of others (6) suggesting that tamoxifen be compared to diethystilbestrol in the treatment of metastatic melanoma, since both appear to have only weak antitumor activity in this disease.

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Ronald R. Reimer

Fred Hutchinson Cancer
Research Center
1124 Columbia St
Seattle, WA 98104

John Costanzi

University of Texas
Medical Center
Galveston, TX 77550

Carol Fabian

University of Kansas
Medical Center
Kansas City, KS 66103

April 12, 1982

Transplacental Passage of Cyclophosphamide[1]

Cyclophosphamide (CP) is an alkylating agent widely used for the therapy of several solid and hematologic malignancies. The drug is a potent mutagenic substance (1), teratogenic in various animal species including man. The obvious risks connected with the use of cytotoxic drugs such as CP during pregnancy may in some cases be outweighed by the benefits to the mother or to both the mother and the fetus.

This was the case when a 33-year-old pregnant woman (gravida II para 1) at the 25th gestational week was diagnosed as having stage IVB Hodgkin's lymphoma. Because the disease was rapidly progressing by the 29th gestational week, chemotherapy with CP (400 mg/m^2, Days 1–5), vincristine (1.4 mg/m^2, Day 1), and prednisone (100 mg/m^2, Days 1–5) (CVP) was started. Fetal retardation was suspected on the basis of serial ultrasound scans, and at the 34th gestational week amniocentesis was performed to assess fetal lung maturity. The lecithin/sphingomyelin ratio was 3, indicating a "mature" fetal lung. Therefore, a cesarean section was performed at the 35th week; the female infant, weighing 2300 g (in the normal range of Italian fetal growth curves; over the 10th percentile), was well and was discharged at 7 days of life.

As amniocentesis was performed 1 hour after the last iv dose of CP (400 mg/m^2) in the second course of chemotherapy, it was possible to analyze the CP concentration in the amniotic fluid. With the informed consent of the mother, maternal blood samples (1.5 ml) were collected in heparinized tubes before and at intervals during the 24 hours following CP treatment. Plasma was obtained by centrifugation. After double extraction at alkaline pH with ethyl acetate, a previously described gas chromatographic method (2) was applied for CP determination after *N*-trifluoroacetylation, using ifosfamide as internal standard. The original method was modified using a nitrogen phosphorus selective detector.

Figure 1 shows plasma CP levels in the mother and CP concentration in the amniotic fluid. The CP level in am-

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[1] Supported in part by a CNR contract in clinical pharmacology and rare diseases by the Italian Association for Cancer Research.

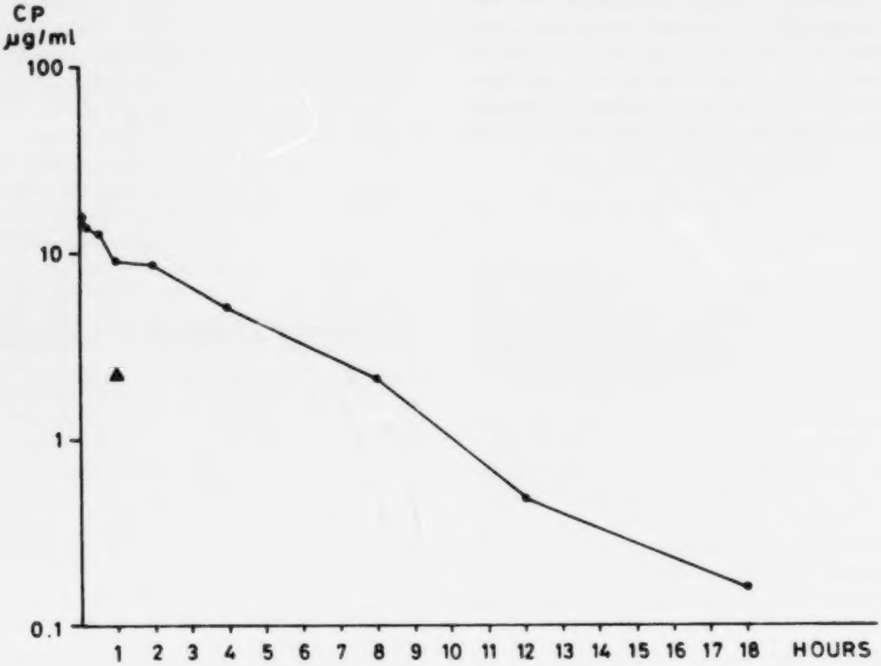

FIGURE 1.—Plasma levels of CP in a pregnant woman given 400 mg/m^2, iv (●). CP in amniotic fluid of the same patient 1 hr after drug administration (▲).

niotic fluid (2.1 µg/ml) was considerable, amounting to approximately 25% of the plasma level at the same time. The disappearance of CP from plasma fits a biexponential pattern of decay as already described for nonpregnant women (3,4). The elimination half-life was 146 minutes, slightly less than previously reported (3,4); the volume of distribution was 0.78 liters/kg, in agreement with previous data (3,4).

As far as we know, this is the first report of transplacental passage of CP. The finding could have considerable practical implications, considering that fetal liver microsomal enzymes contain a P-450-dependent mono-oxygenase system (5) and therefore can presumably activate CP to the mutagenic and cytotoxic metabolites hydroxycyclophosphamide, aldophosphamide, and phosphoramide mustard.

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M. D'Incalci*
C. Sessa
N. Colombo

Istituto di Ricerche Farmacologiche "Mario Negri"
Milan, Italy

G. de Palo

Istituto Nazionale per lo Studio e la Cura dei Tumori
Milan, Italy

A. E. Semprini
G. Pardi

I Clinica Ostetrica Ginecologica
Università di Milano
Milan, Italy

April 14, 1982

**Reprint requests to:* M. D'Incalci, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

Aclacinomycin A Flare

In an article in the November/December 1979 issue of *Cancer Treatment Reports* (vol 63, pp 2067–2069), Nicholas J. Vogelzang described an "Adriamycin (doxorubicin) flare" skin reaction that occurred in 3% of 100 consecutive patients observed. At the Ohio State University Hospital, a similar reaction has been observed in one patient receiving aclacinomycin A, a phase I drug similar to doxorubicin in structure (1). This one occurrence of a flare reaction is the only one of its kind noted after 110 administrations of aclacinomycin A to 54 patients at Ohio State University Hospital.

The aclacinomycin A flare was noted in a 67-year-old white female with metastatic gastric carcinoma. She had received previous chemotherapy with 5-FU, doxorubicin, and mitomycin (October 1979 to April 1980) and with two courses of AMSA (October 1980); the flare reaction occurred during her sixth course of treatment with aclacinomycin A. She had had no previous reactions to past doses of aclacinomycin A ranging from 90 mg to 162 mg and had had a total dose of 572 mg in previous treatments. The sixth course of aclacinomycin A given on June 25, 1981, was her last. She died 10 days later of respiratory arrest.

The clinical nurse specialist administering the drug started an iv infusion of 5% dextrose in water with a 23-gauge scalp vein butterfly needle in the median vein of the right forearm. The patency of the line was documented before, during, and after the administration of the drug by occlusion of the vein proximal to the needle and by observation of a good blood return on aspiration with a syringe and needle inserted through the bulb of the iv tubing. The patient was instructed to report any pain or discomfort at the venipuncture site while 90 mg of aclacinomycin A was given by slow iv push into the continuously flowing iv infusion of 5% dextrose in water. The drug was administered in approximately 10 minutes. About 5 minutes into the infusion, the patient complained of a stinging sensation about 4 inches proximal to the venipuncture site. The nurse then slowed the pushing of the aclacinomycin A and allowed longer intervals of flushing the line with 5% dextrose in water. The stinging lessened somewhat but persisted throughout the rest of the infusion. There were no physical changes noted at the location of the stinging sensation. No redness, swelling, pain, or other discomfort was experienced at the venipuncture site. However, after the aclacinomycin A had been administered, some red, nonraised blotches began to appear along the vein from the venipuncture site to the area where the patient had complained of the stinging. The infusion of 5% dextrose in water was continued at the full rate, and a heat lamp was placed over the affected area (this is our oncology unit's usual procedure with vein irritation from drugs). The stinging stopped shortly, but it took about 1 hour before the red blotches subsided and finally disappeared completely.

In conclusion, it appears that aclacinomycin A may cause a flare reaction similar to that of doxorubicin.

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Elaine C. Glass

Interdisciplinary Oncology Unit
The Ohio State University Comprehensive Cancer Center
Columbus, OH 43210

April 14, 1982

Leukemia Occurring After Treatment of Small Cell Lung Cancer [1]

Since the advent of effective combination chemotherapy, the median survival of patients with small cell carcinoma of the lung has reached 10–12 months. Few patients, however, survive beyond 2.5 years (1). Between 1974 and 1978, 40 patients at the University of Minnesota were treated with lomustine (CCNU)- and doxorubicin-containing regimens (2). One patient survived 5 years, and he has developed acute leukemia.

CASE REPORT

This 56-year-old white male smoker was well until December 1976, when he developed chest pain, cough, and hoarseness. Bronchoscopy revealed a fungating tumor mass extending distally from the lateral wall of the left main stem bronchus. Pathologic review at the University of Minnesota confirmed the diagnosis of small cell anaplastic carcinoma. The physical examination was normal. The chest X-ray disclosed a left upper lobe infiltrate and a left hilar mass. The gamma glutamyl transferase was slightly elevated, but the liver and spleen scan was normal. The level of carcinoembryonic antigen was elevated (17 ng/ml) at the start of therapy. The bone marrow examination was normal except for increased iron stores. The HLA type was A2A3 B7BW15.

Chemotherapy was begun on December 31, 1976. The patient received 100 mg/m^2 of CCNU, 50 mg/m^2 of doxorubicin, 1 mg/m^2 of vincristine, and 100 mg/m^2 of procarbazine orally daily for 14 days. After the first course of chemotherapy, the chest radiograph was normal. The patient was treated with identical doses of drugs on February 11, 1977, and March 29, 1977. During the fourth course of chemotherapy beginning May 15, 1977, he received 4000 rads via a 10 MeV linear accelerator to the mediastinal, hilar, and left upper lobe areas. He also received 120 mg of CCNU and 2 mg of vincristine coincident with the radiotherapy. The patient received chemotherapy in varying doses until November 6, 1978. By that time he had received a total of 870 mg of doxorubicin, 1300 mg of CCNU, 17 mg of vincristine, and 14 g of procarbazine.

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[1] Supported in part by Public Health Service grant CA-19527 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; by American Cancer Society Junior Faculty Fellowship JFCF 639 to N. J. Vogelzang; by the Minnesota Medical Foundation; and by the Masonic Memorial Hospital Fund, Inc.

The patient was observed after completion of therapy, and progressive thrombocytopenia was noted. In January 1981, the platelet count was 91,000/μl. On September 28, 1981, a platelet count of 17,000/μl, easy bruisability and recurrent skin infections were noted. The peripheral wbc count was 3200/μl, with 3% blast cells, 52% neutrophils, 18% lymphocytes, 25% monocytes, and 2% eosinophils. The hemoglobin was 11.2 g/100 ml. The carcinoembryonic antigen level was normal. The bone marrow examination revealed dyserythropoiesis, ringed sideroblasts, decreased megakaryocytes, and 16% myeloblasts. The blast cells were large, with variable amounts of cytoplasm and myeloperoxidase-positive granules. No Auer rods were seen. The morphology of these blast cells was consistent with the M2 subtype of acute leukemia using the French-American-British classification.

G-banded chromosomes were analyzed in 23 cells from direct preparations and 24-hour cultures of bone marrow. Six cells showed a normal 46, XY male karyotype. The remaining 17 cells had a modal number of 46 chromosomes with a duplication of the long arm of one No. 12 chromosome as the only abnormality: 46, XY, dup (12) (q 12→q 24).

The patient elected to have only transfusion therapy. Since the diagnosis of leukemia, he has required eight to 16 HLA-matched donor platelet packs per week. He has had no infections and has not required rbc transfusions. A repeat bone marrow on December 14, 1981, disclosed 15.8% myeloblasts.

DISCUSSION

Patients with small cell lung cancer who are long-term survivors after intensive multimodality therapy appear to be at risk for treatment-related leukemia (3,4). The degree of risk is unclear. Hansen et al (5) have not observed any treatment-related leukemia in 16 long-term survivors of multimodality treatment. Matthews et al (1) reported no cases of chemotherapy-related leukemia in 46 patients followed for > 2.5 years. Bradley et al (4) reported on eight long-term survivors, one of whom developed erythroleukemia. Multiple cytogenetic abnormalities were found in the malignant blast cells.

Specific chromosome abnormalities that have been reported to be associated with treatment-induced acute leukemia include partial deletion of the long arm or complete loss of chromosomes 5 and 7 (6). The significance of the duplication found in this patient is unknown.

Finally, the occurrence of acute leukemia in this and other patients should stimulate a reappraisal of the dose, schedule, and role of leukemogenic agents such as CCNU, procarbazine, radiotherapy, and alkylating agents in the treatment of small cell lung cancer. The length and intensity of induction therapy need close scrutiny, and agents with less leukemogenic potential, such as etoposide and doxorubicin, should find wider use.

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Nicholas J. Vogelzang*
Diane Arthur
B. J. Kennedy

Department of Medicine
Section of Medical Oncology
and the Department of Laboratory Medicine and Pathology
University of Minnesota Health Sciences Center
Minneapolis, MN 55455

April 26, 1982

**Present address:* Section of Hematology/Oncology, University of Chicago, Box 420, 950 E 59th St, Chicago, IL 60637.

ANNOUNCEMENTS

Multidisciplinary Approach to Management of Patients With Mesothelioma

East Lansing, Michigan, September 16–17, 1982

This international workshop is sponsored by The International Association for Study of Lung Cancer and the Michigan State University, in cooperation with the Ingham Medical Center. A practical multidisplinary approach to the management of patients with malignant mesothelioma will be outlined, and a master protocol for the collection of data on environmental factors and treatments associated with particular patients will be designed. A line of communication for exchange of information between participating investigators will also be planned.

The number of participants is limited.

For further information, contact: M. A. Hollandsworth, Asbestos-Mesothelioma Program, 405 W Greenlawn Ave, Suite 330, Lansing, MI 48909. Telephone (517) 374-2107.

Urologic Cancer Course

Boston, Massachusetts, September 22–24, 1982

This intensive 3-day course, to be held at the Parker House Hotel, will provide an overview of the clinical and pathophysiologic basis for the diagnosis and treatment of patients with urologic cancer. The course will emphasize the multidisciplinary approach that constitutes the foundation of contemporary care for patients with urologic cancers. Principles derived from the disciplines of urologic surgery, medical oncology, and radiation therapy will be highlighted and incorporated into a comprehensive consideration of therapeutic practices. Although teaching will be primarily provided through lectures, panel discussions addressing major current controversies in urologic cancer will be an integral portion of the course. A comprehensive syllabus will be provided.

Guest faculty will include Drs. Lawrence H. Einhorn, Donald Skinner, Willet F. Whitmore, Jr, and Leonard Zinman. The Harvard faculty participants will include leaders in urologic cancer from the Sidney Farber Cancer Institute, Brigham & Women's Hospital, Joint Center for Radiation Therapy, Children's Hospital Medical Center, Beth Israel Hospital, and Massachusetts General Hospital.

The course fee is $325.00

For further information contact: Harvard Medical School, Department of Continuing Education, Boston, MA 02115 (617-732-1525).

Cancer and Nutrition, an Oncology Update at the 15th Annual Malignant Disease Symposium

Chapel Hill, North Carolina, October 22–23, 1982

The Clinical Oncology Education Program and the Cancer Research Center at the University of North Carolina School of Medicine at Chapel Hill will sponsor this 2-day symposium. It will cover the mounting evidence from current epidemiologic and clinical research studies that points to an association of nutrition with certain cancers and how new preventions and treatments against these cancers are being tested as a result of these studies. In addition, new techniques for feeding by mouth, tube, and vein and how they are changing the management of cancer patients will be discussed.

This symposium has been organized to bring the most up-to-date information about these developments to practicing physicians, house staff, nurses, and health professionals involved with cancer patients.

The registration fee is $125; the symposium offers 12 credit hours, Category 1 AMA-PRA.

For further information, contact: William D. Heizer, MD, Cancer Research Center, Box 30, MacNider Bldg, Chapel Hill, NC 27514.

Paraneoplasia: Basic Concepts and Clinical Aspects

Brussels, Belgium, November 5–6, 1982

This 2-day symposium presented by the European Organization for Research on Treatment of Cancer will focus on endocrine paraneoplasia, neurologic paraneoplasia, coagulopathies, and nonhormonal tumor markers. The Chairmen are J. Waldenström and H. J. Tagnon.

In addition, free communications of 10 minutes each will be accepted. An abstract must be sent to the address below before September 30, 1982. Seating is limited and preregistration is recommended.

For further information, contact: Dr. M. Staquet, Director, EORTC Data Center, rue Héger-Bordet 1, 1000 Brussels, Belgium. Telephone: (2) 538.57.90, Telex: 22773.

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

☆U.S. GOVERNMENT PRINTING OFFICE: 1982 361-140/2 1-3

a publication
of the
national cancer institute

NIH Publication No. 82-22

66/9

1982

cancer treatment reports

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September 1982
Volume 66
Number 9
Pages 1687-1789

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

HE 20.3168:

cancer treatment reports

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Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. A second table will be permitted only when requested by the editorial board. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

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1787 ANNOUNCEMENTS

Chemotherapy for Osteogenic Sarcoma: An Investigative Method, Not a Recipe[1]

Gerald Rosen* and Anita Nirenberg[2]

The controversy over the role of chemotherapy for the treatment of osteogenic sarcoma arises because osteogenic sarcoma has been in the past and always will be a difficult and resistant tumor to treat with chemotherapy. In the majority of instances, the various protocols that have recently proliferated for the treatment of osteogenic sarcoma have been culled from the literature and put together based on theoretic considerations. Whereas this may suffice for the treatment of leukemias and other chemotherapy-sensitive diseases, chemotherapy for osteogenic sarcoma was hard to develop and is rather unique. Unfortunately, dose schedules must be precise with little variation, since experience has shown that the treatment of metastatic osteogenic sarcoma with high-dose methotrexate and leucovorin rescue can be successful only if it is administered in exactly the way that experience has dictated objective responses will occur.

The major breakthrough in the chemotherapy for osteogenic sarcoma is attributed to the work of Jaffe and Paed (1) in 1972 when they showed that massive doses of methotrexate with leucovorin rescue could cause metastatic osteogenic sarcoma to regress. The usual dose initially found to be effective was about 300 mg/kg. Kim and Djerassi (2) suggested that metastatic osteogenic sarcoma might respond to this unique treatment and that there is a threshold dose of methotrexate above which responses occur and below which there is absolutely no response. The use of many other chemotherapeutic agents in other tumors has shown us that even with suboptimal doses there is some partial response to treatment, and more complete responses may be obtained at higher doses. However, the majority of other agents have dose-limiting toxicity. The unique aspect of high-dose methotrexate with leucovorin rescue is that while even small, suboptimal doses such as 50, 100, and 200 mg/kg are potentially as lethally toxic as 300 or 400 mg/kg, it may only be the latter doses that can affect this disease in younger children.

This observation by Kim and Djerassi has been confirmed by our experience at the Memorial Sloan-Kettering Cancer Center, where we have given high-dose methotrexate and leucovorin rescue to > 100 patients with evaluable metastatic disease and > 200 patients with evaluable primary tumors. Experience in the direct observation of patients with evaluable disease who received this treatment has allowed us to determine the optimal dose of high-dose methotrexate for each patient. In addition, extensive pharmacokinetic studies of patients receiving high-dose methotrexate have allowed us to define predictive indicators of impending toxic effects and the medical intervention necessary to prevent severe or even lethal toxic effects (3,4). We have found that it is not enough to ensure the safety of high-dose methotrexate treatment; it is equally important to ensure the effectiveness of treatment. Otherwise, patients may become just as dead from metastatic disease as they would be from severe, lethal toxic effects.

We have reported a 40% complete response rate of osteogenic sarcoma to single-agent, high-dose methotrexate with leucovorin rescue. The overall partial and complete response rate was 68% (5). The doses and scheduling used for these studies were arrived at during 5 years of experience in treating > 200 patients with evaluable disease, with approximately 5000 high-dose methotrexate treatments.

The effective dose of methotrexate was 8 g/m^2 in fully grown adolescents and 12 g/m^2 in younger children who had not reached their adult stature. In addition, pharmacokinetic data have shown that 24-hour methotrexate levels in the range of 10^{-5} M seem to correlate with effectiveness of treatment and that 72-hour serum methotrexate levels $> 10^{-7}$ M can be potentially toxic if leucovorin is not continued until the serum methotrexate level falls below 10^{-7} M. We accomplish the repetition of these safe and effective pharmacokinetics by performing outpatient treatment. We have found that the patient and family are much more reliable in recording and obtaining the correct urine output than are hospital staff. The alkalinization of the urine for the first 24 hours has also prevented renal toxicity, which can lead to prolonged elevated methotrexate levels; the abrogation of the effect of methotrexate on the tumor, due to the need for elevated and prolonged leucovorin administration; and the inability to resume treatment on time. When the patient is sent home following high-dose methotrexate treatment, he and/or his parent checks

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[1] Received Mar 22, 1982; accepted Apr 8, 1982.

[2] Departments of Pediatrics (*G. Rosen* and *A. Nirenberg*) and Medicine (*G. Rosen*), Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Gerald Rosen, MD, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

the urine at every voiding, and if the pH is not ≥ 7.0, additional oral sodium bicarbonate is administered. This is difficult to do with inpatient treatment, since busy nurses in the evening and night cannot be expected to immediately check the pH for every patient who voids and immediately administer bicarbonate. Frequently, an acid urine from an inpatient may not come to the nurse's attention for 1–2 hours, and it may be another half hour before the patient receives bicarbonate. This delay alone can be responsible for renal damage and potential severe toxic effects.

To produce the pharmacokinetics of methotrexate elimination that we feel is both safe and effective, patients are instructed, for the first day following high-dose methotrexate, to have a 24-hour urine output of 1500–1600 ml/m^2 of alkaline urine (fig 1). For the next 2 successive days they are instructed to have a urine output > 2000 ml/m^2. In most outpatients who have received extensive training, the urine output is within 100–200 ml of the recommended guidelines.

In these circumstances, we have found high-dose methotrexate with leucovorin rescue to be extremely

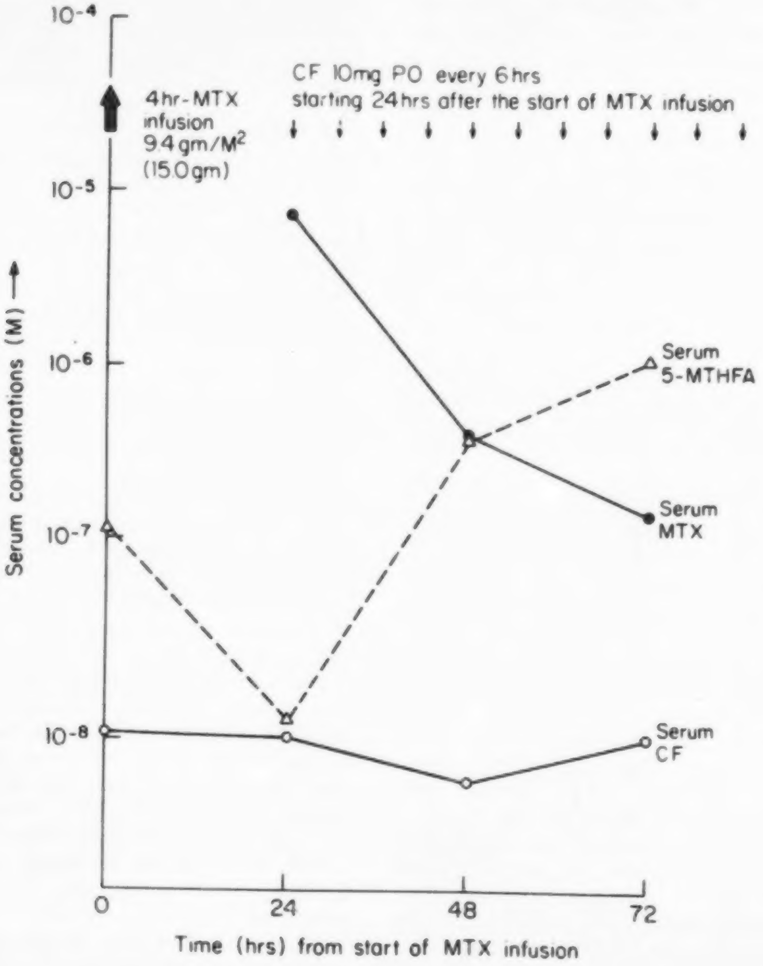

FIGURE 1.—Typical pharmacokinetic profile of serum methotrexate (MTX) and serum 5-methyltetrahydrofolate (5-MTHFA) levels following 15 g of high-dose MTX and 10 mg of leucovorin rescue (citrovorum factor, CF) orally every 6 hrs starting 24 hrs after the start of MTX infusion. The serum MTX level is about 1×10^{-5} M at 24 hrs and about 1×10^{-7} M at 72 hrs. Since CF is converted to 5-MTHFA as it is absorbed in the gastrointestinal tract, CF cannot be measured accurately in the serum. When the oral dosage of CF is 10 mg every 6 hrs, MTX and 5-MTHFA become equimolar in the serum prior to 48 hrs. With these pharmacokinetics, treatment is usually effective and produces no toxicity (*see* fig 2).

effective in the treatment of osteogenic sarcoma (fig 2). Furthermore, we have frequently noted that when hospitalization is required for medical conditions that would not permit the outpatient administration of high-dose methotrexate, a higher percentage of patients fail to respond to therapy and there is a higher incidence of toxic effects.

We have extensively reported on the value of high-dose methotrexate with leucovorin rescue in the treatment of osteogenic sarcoma. Yet no single investigative protocol at another cancer center calls for the use of high-dose methotrexate as we administer it in the outpatient department to reproduce the pharmacokinetics that we have demonstrated to be both safe and effective (figs 1 and 2). The majority of protocols using high-dose methotrexate with leucovorin rescue not only require hospitalization of the patient but specifically call for increased prehydration with iv fluid prior to and following methotrexate infusion. This is directly contradictory to our experience in the effectiveness of high-dose methotrexate in the treatment of osteogenic sarcoma. Giving the treatment as we have described requires 3 days of extensive followup and diligent attention to daily serum methotrexate levels, urine output, and physical condition. If investigators are not willing to wait (sometimes) until 7:00 PM on Friday evening for the biochemistry laboratory to report that day's 72-hour methotrexate levels and to call patients with levels > 10^{-7} M instructing them to continue taking leucovorin, there will be excessive toxic effects with the use of high-dose methotrexate in the outpatient setting. Because of this requirement, we have administered high-dose methotrexate only on Mondays and Tuesdays so a 3-day followup for the serum methotrexate levels could be done on a daily basis to ensure the safety of treatment.

Our first experience in the adjuvant treatment of 54 patients utilized high-dose methotrexate at the dose of 200 mg/kg, doxorubicin at a dose of 90 mg/m^2, and cyclophosphamide at a dose of 40 mg/kg (1200 mg/m^2). The method of administration for this protocol (T-4) is very similar to that for many protocols used in recent years. The overall continuous disease-free survival of patients who were treated with this protocol was only 50%, again comparable to the results being obtained today with other investigative protocols (6,7). The majority of patients treated had not achieved their full adult stature, and we found retrospectively that they had not received effective doses of high-dose methotrexate; that was the area in need of improvement.

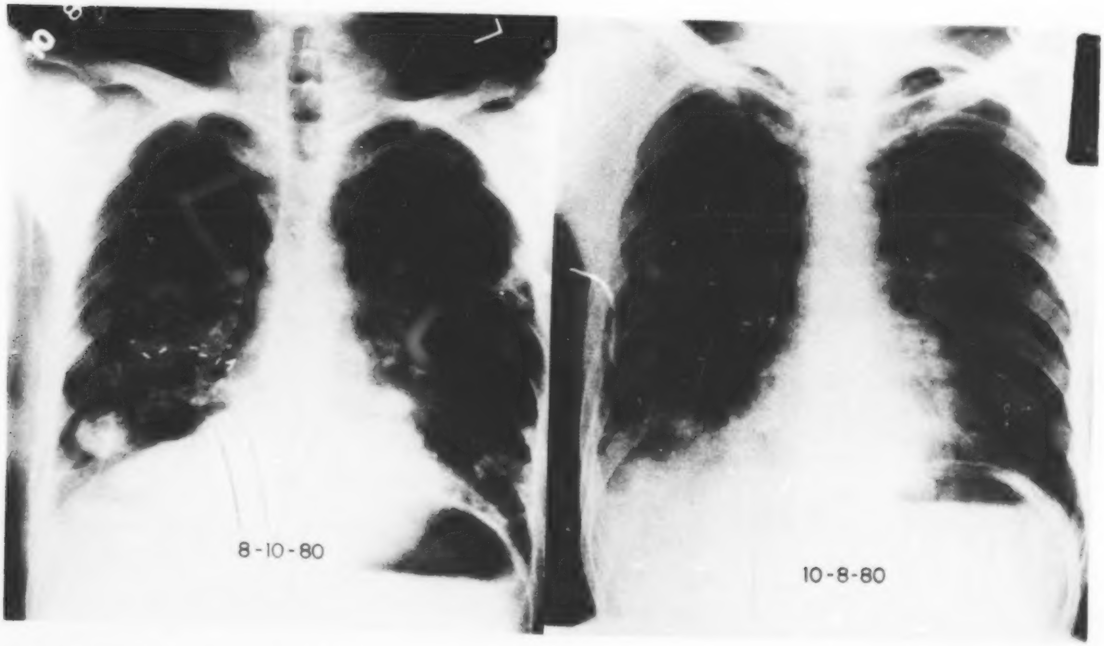

FIGURE 2.—Serial X-rays of a patient who had progressive pulmonary disease while receiving high-dose MTX (15 g) with excessive iv fluid hydration. After referral to Sloan-Kettering he was re-treated at the same dose without excessive fluid hydration; he had a partial response, with a decrease in the size of the large retrocardiac mass as well as other pulmonary metastases.

Further treatment of patients who relapsed with higher doses of methotrexate (12 g/m^2) frequently caused regression of their pulmonary metastases (fig 3).

It was through the use of chemotherapy prior to surgery for osteogenic sarcoma, which was given to make time for construction of an endoprosthesis for limb-salvage surgery, that we were able to observe regression in some primary osteogenic sarcomas when the dose of methotrexate was escalated by 2–4-g increments. The recommendation for the dose of 12 g/m^2 for patients who are not fully grown was made because of that early experience. The observations were also made that no patient responded to a < 12-g dose of high-dose methotrexate and that the escalation to > 20-g doses of high-dose methotrexate seldom yielded any meaningful clinical responses when patients had failed to respond to doses of 16 and 18 g.

These observations and a new dose schedule were incorporated into our T-7 chemotherapy protocol in 1976 (8). In addition, the combination of bleomycin, cyclophosphamide, and dactinomycin (BCD), which had been shown to be effective in the treatment of metastatic osteogenic sarcoma, also replaced the single agent cyclophosphamide. BCD chemotherapy has not been used in any other protocol to date, although we have found it extremely effective, yielding a response rate of approximately 60% (higher than that produced with doxorubicin as a single agent) and with less toxicity than is observed following the use of doxorubicin at the recommended dose of 90 mg/m^2/course (9). We had achieved 82% continuous disease-free survival for all patients treated with the T-7 chemotherapy protocol. All patients treated with that protocol have now been followed for 40–66 months (median, 52) from the start of treatment. The 82% represents continuous disease-free survival and does not include patients who relapsed and were subsequently salvaged with thoracotomy and continued chemotherapy.

Through the use of preoperative chemotherapy and the evaluation of its effect on the primary tumor, we had determined that all patients having a histologically confirmed complete or near-complete (> 90%) response of the primary tumor following the administration of preoperative chemotherapy became continuous dis-

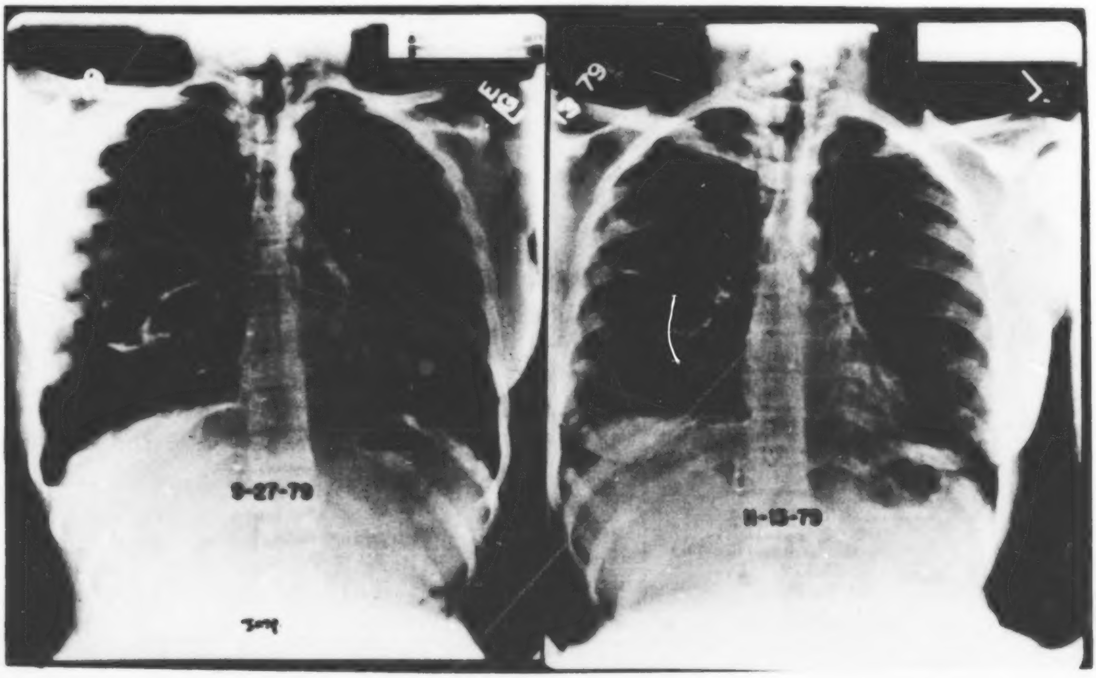

FIGURE 3.—This 13-yr-old girl developed bilateral pulmonary metastases while being treated with a Southwest Oncology Group adjuvant chemotherapy protocol consisting of 3 doses of high-dose MTX (4, 8, and 10 g) interspersed with 2 courses of doxorubicin during 3 mos. She was referred to our clinic for treatment. Note complete disappearance of pulmonary metastases following 4 weekly doses of MTX (14 g; 12 g/m^2).

ease-free survivors, with the exception of one patient who relapsed with a local recurrence after inadequate resection of the primary tumor. All of the relapses occurred in patients whose primary tumor did not respond to preoperative chemotherapy following histologic evaluation of the resected primary tumor (6,8,10).

In 1978, a new protocol (T-10) called for the identification of these poor responders to high-dose methotrexate with leucovorin rescue and other preoperative chemotherapy and the addition of cisplatin to their adjuvant chemotherapy protocol following surgery after high-dose methotrexate was discontinued. This therapeutic maneuver proved to be highly significant in terms of increasing the continuous disease-free survival of patients who did not respond well to chemotherapy prior to surgery. It further raised the continuous disease-free survival of the entire group of (now) 79 patients to > 90% (6–40 months' followup; median, 24) (10).

The majority of patients failing to have a good response to high-dose methotrexate chemotherapy prior to surgery had partial responses; therefore, the addition of cisplatin and continued doxorubicin and BCD following surgery in this group of patients was therapy in addition to the preoperative high-dose methotrexate, which did cause approximately 50% tumor regression in the majority of patients. High-dose methotrexate is probably still necessary in that group of patients since there is some response to it. Further information has shown that the use of cisplatin and doxorubicin alone without prior high-dose methotrexate has led again only to a 52% disease-free survival rate (L. Ettinger, personal communication).

It has recently been claimed that osteogenic sarcoma is becoming a more benign disease (11,12). That has not been our experience (13) nor the experience of other large groups (14,15) studying the natural history of osteogenic sarcoma. However, even if the disease-free survival is between 30% and 40% with surgery alone, it is still far below that which can be achieved with optimal chemotherapy (10). No one would have second thoughts about using adjuvant chemotherapy in diseases that have a 50% cure rate for surgery alone. A one out of two chance of dying of disease in 5 years is unacceptable; patients with tumors such as primary malignant fibrous histiocytoma of soft tissue, with a cure rate following surgery alone that might be as high as 50%, are offered adjuvant chemotherapy, and this is generally accepted by the oncology community as being proper treatment. In addition, the higher cure rate following surgery alone for osteogenic sarcoma is probably related to the earlier diagnosis of small pulmonary metastases by computerized tomographic scan, and those patients are eliminated from the study group. It is precisely those patients who need effective chemotherapy, or the survival rate for that subset of patients will be zero following surgery alone.

Why, then, is there controversy over the treatment of osteogenic sarcoma even if the cure rate can be as high as 40% with surgery alone? The reason is that many investigators have been frustrated in their attempts to successfully treat osteogenic sarcoma and have concluded that if they cannot treat it effectively the treatment is of no value, without stopping to think of how the treatment procedure may be made more effective. Safe and effective high-dose methotrexate treatment is not merely the giving of a drug, it is a pharmacokinetic procedure. It is not an art, it is a precisely spelled-out science based on precise data obtained from each patient following each treatment. Based on the data obtained in this biologically varying milieu, the procedure may have to vary.

At the Memorial Sloan-Kettering Cancer Center, Dr. Ralph Marcove was the first surgical oncologist to employ limb-salvage surgery in the treatment of osteogenic sarcoma (6). In the past, people have commented that our results must be so good because our referral pattern reflects the selection of patients with resectable lesions. This could not be further from the truth of the real situation. It is well-known that by the time patients are referred to tertiary treatment centers they usually have tumors that are further advanced than patients who are treated in regional university centers in their home town. In general, our patients have presented with more and more advanced disease over the years, as has been determined by examining the X-rays and bone scans and the extent of the primary lesion in all of our patients. To date, all but two of 198 protocol-treated patients have presented with moderate to large soft tissue extension of the primary tumor.

In our first chemotherapy protocol (T-4 and T-5, referred to as T-4 for the sake of uniformity), 58% of patients underwent limb-salvage procedures and the rest had amputations (6). In our current protocol (T-10), only 48% of patients have been able to have successful resections (10). We have made chemotherapy prior to surgery much more effective in shrinking primary tumors over the past 8 years, and the surgical techniques have improved as well as the skill and daring of the surgical oncologist in performing limb-salvage procedures. However, the lower proportion of patients who were able to have resection over the past 8 years is a primary indication that patients referred to this tertiary cancer treatment center have more advanced lesions than patients had years ago and that they have more advanced disease than that seen at regional medical centers where the initial biopsy has been performed and definitive treatment started.

The surgical cure rates recently claimed for osteogenic sarcoma without chemotherapy may be artificially high; to determine the effect of chemotherapy on osteogenic sarcoma it is necessary to define subsets of patients with similar prognoses. The most significant prognostic variable that we have identified to date in the survival of patients with osteogenic sarcoma is the location of the primary lesion. If all of the extremity lesions are considered, it can be seen and is well accepted that patients with primary femur lesions have a worse prognosis than patients with primary lesions of the humerus and tibia. Thus, if a series is weighted with patients who have tibia or humerus lesions, there will be a higher cure rate following surgery alone. In addition, it has been claimed that osteogenic sarcoma patients > 21 years old have a better prognosis. That has not been our experience, but for the sake of uniformity, a specific age group and perhaps a specific site should be designated when disease-free survival rates for osteogenic sarcoma are reported.

During the past 8 years we have treated 185 patients with primary osteogenic sarcoma of the extremity in the age group ≤ 21 years. Ninety-six (> 50%) of the patients had lesions of the femur. In regard to patients with femur lesions alone (fig 4), the continuous disease-free survival of the first 32 patients treated with our T-4 protocol is not much different from that being obtained with various treatment protocols throughout the country at this time (38%) (17-21). However, with refinements in the administration of high-dose methotrexate, in particular the dose of 12 g/m^2 for younger adolescents and children, the continuous disease-free survival rate for patients with femur lesions rose to 65% with the T-7 chemotherapy protocol. With the addition of cisplatin to the treatment of patients not having a complete response to chemotherapy with high-dose methotrexate prior to surgery, the continuous disease-free survival rate for patients with femur lesions rose to 88% with the T-10 chemotherapy protocol. These data include as failures 11 patients (five receiving the T-4 chemotherapy protocol, four receiving the T-7 chemotherapy protocol, and two receiving the T-10 chemotherapy protocol) who failed treatment due to local recurrences in the extremity following what was, retrospectively, inadequate limb-salvage surgery. If one excluded those surgical failures, the continuous disease-free survival rate for each respective protocol would be even higher.

There are those who claim that > 70% of patients can be free of disease at a median time of approximately 2 years with surgery alone, thoracotomy to remove pulmonary metastases, and then delayed chemotherapy (22). In figure 5, the overall survival (not continuous disease-free survival) of our patients is plotted for each of the various protocols; 70% of the patients with femur lesions who received our old T-4 chemotherapy protocol (which we do not consider good chemotherapy at the present time) were surviving at 2 years. Practically all of them were disease-free at that time, since when patients relapsed they had pulmonary metastases removed at thoracotomy; however, they were not continuously free of disease, and many relapsed and died during the next 5 years. These data are as good as, if not better than, any data about surgical treatment for osteogenic sarcoma, since they represent only patients with primary femur lesions, who have a worse prognosis than patients with primary sites in other extremity bones. However, the survival rate of these patients gradually decreased to 37.5% at 7 years, which is not significantly better than the continuous disease-free survival rate of the patients receiving the T-4 chemotherapy protocol (compare fig 5 with fig 4).

We have found that through the use of multiple thoracotomies and continued chemotherapy, it is possible to extend the survival of patients with metastatic disease to between 5 and 8 years from the onset of pulmonary metastases; however, most patients will eventually succumb to recurrent pulmonary metastasis or other distant metastases. To consider the results of surgery alone (including thoracotomy), with chemotherapy added only after pulmonary metastases are resected, the 5- and 6-year disease-free survival rates must be examined. We have found that the continuous disease-free survival rate is the closest correlation to the eventual 6- and 7-year survival rates.

In regard to osteogenic sarcoma in sites other than the femur, figures 6 and 7 show that patients with tibia lesions and humerus lesions seem to have a good prognosis regardless of the chemotherapy protocol used; however, the number of patients treated with the T-4 chemotherapy protocol is too small to say that there are significant differences between the results in T-4-treated patients and the results in patients treated with what we now consider much improved chemotherapy (T-7 and T-10). Nevertheless, the overall continuous disease-free survival rates for both groups of patients are 87% and 88%, respectively, with a 94% continuous disease-free survival rate for patients with tibia and humerus lesions treated with the current T-10 chemotherapy protocol.

One patient treated with the T-4 chemotherapy protocol had a late relapse (after 6 years). He was treated with en bloc resection of a primary osteogenic sarcoma of the tibia, and although not yet proven, it is suspected that a local recurrence may have been the reason for his late failure.

The 185 patients reported above represent all of the patients with primary osteogenic sarcoma of an extremity referred to our service during the past 8 years, except:

(*a*) five patients who presented with simultaneous bone metastases,

(*b*) 27 patients who presented with simultaneous pulmonary metastases,

(*c*) three patients who presented with small cell histology (treated on the Ewing's sarcoma protocol),

(*d*) 22 patients who presented with primary osteo-

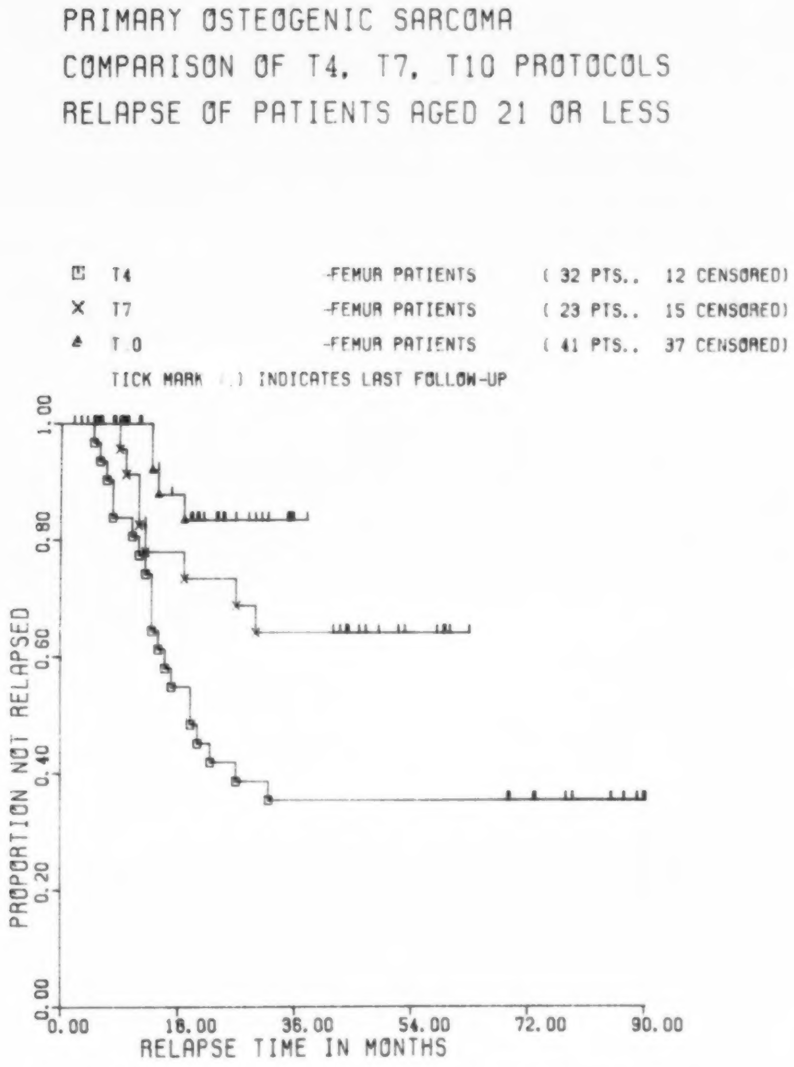

FIGURE 4.—Kaplan-Meier (16) plots of the continuous disease-free survival (DFS) of 96 patients with primary osteogenic sarcoma of the femur, treated on 3 successive protocols over a period of 8 yrs. Censored = No. of patients continuously free of disease. Included with treatment failures are patients with local recurrences following resection (T-4, 5 patients; T-7, 4 patients; and T-10, 2 patients). Overall continuous DFS is projected at 62.5%. Use of 12 g/m² of MTX for young children and addition of BCD account for the statistically significant difference in continuous DFS between T-4 and T-7 protocols. Addition of cisplatin for patients not responding to high-dose MTX accounts for the increase in continuous DFS from 65% (T-7) to the projected 3-yr DFS of 82.5% (± 7.0%) with the T-10 protocol.

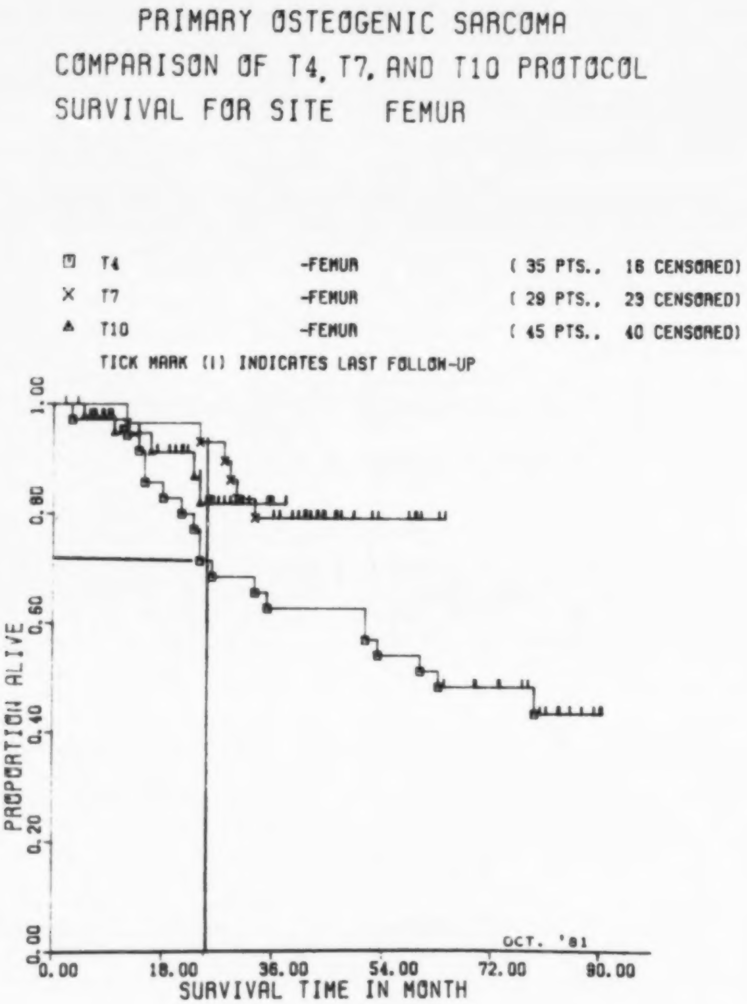

FIGURE 5.—Kaplan-Meier plots of overall survival of patients with primary osteogenic sarcoma of the femur treated with 3 different chemotherapy protocols. The 7-yr survival of the T-4 group is not significantly different from the continuous DFS rate noted in fig 4.

genic sarcoma of an extremity > 21 years old, and

(e) three patients who refused to have their initial four high-dose methotrexate treatments at this center and returned to their referring center for treatment.

The requirement that patients receive their initial high-dose methotrexate treatments at this center was included in the eligibility criteria since we believed it was our responsibility to adequately train patients as to what medical intervention may be necessary so that the high-dose methotrexate treatment could be safely and effectively administered in the outpatient department. Because we insisted on this minimal criterion, we have had no patient deaths due to high-dose methotrexate treatment in the past 5 years, representing > 5000 high-dose methotrexate treatments.

It should be noted that in addition to the above exclusions, three patients presenting with low-grade histology were excluded from treatment. On the other hand, three patients whose initial biopsies appeared to show low-grade osteogenic sarcoma later received adjuvant

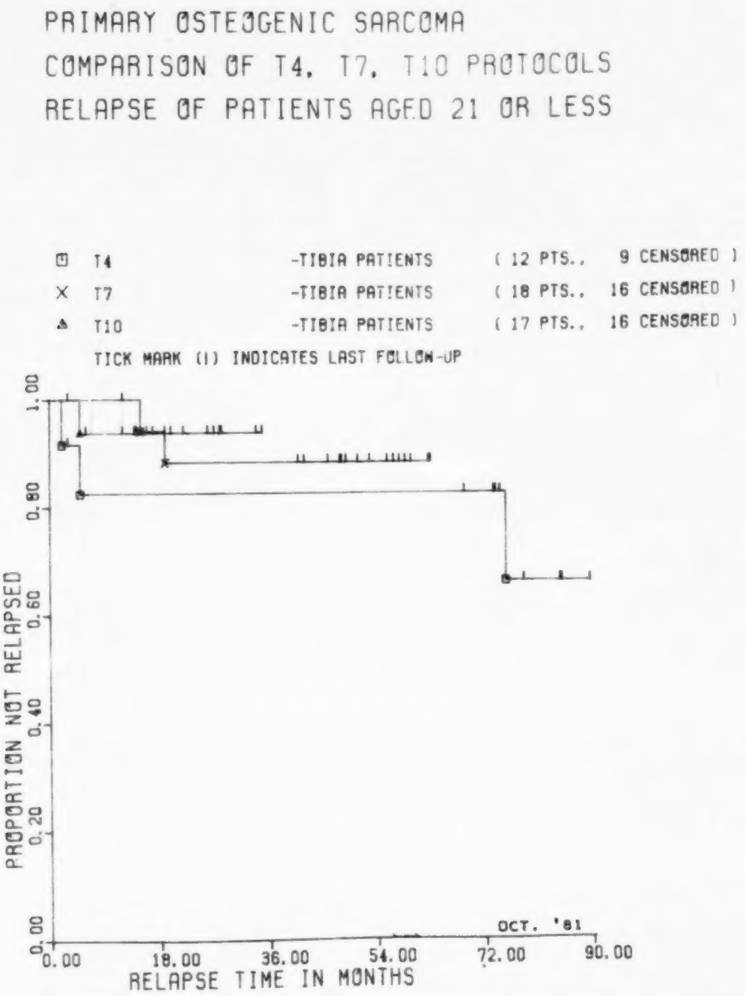

FIGURE 6.—Kaplan-Meier plots of the continuous DFS of patients with primary osteogenic sarcoma of the tibia. Because of the small number of patients treated with the T-4 protocol and the very high continuous DFS rates, there is no statistically significant difference among patients treated with the 3 different protocols.

chemotherapy when high-grade, fully malignant, osteogenic sarcoma was found in the resected or amputated specimen. All 185 patients who were treated with the chemotherapy protocol had osteogenic sarcoma of fully malignant histology (grades III–IV).

From the data presented above, it should be perfectly clear that chemotherapy is effective in increasing the cure rate for osteogenic sarcoma, a malignant neoplasm that in the past has been difficult to treat with chemotherapy but has recently been sensitive to the precisely defined modalities of treatment discussed above. The highest chance for cure in a patient with a malignant neoplasm is achieved through the use of adjuvant chemotherapy prior to the time the patient relapses (23). In addition, the selection of high-risk patients on the basis of their poor histologic response to chemotherapy prior to surgery allows us to institute alternative chemotherapy prior to the patient relapsing, again, at the time when the chance of increasing the cure rate is optimal. This has allowed a further increment in the cure rate for osteogenic sarcoma in our current T-10 chemotherapy study (10).

People have argued that the chemotherapy for osteogenic sarcoma is extremely expensive. This is based

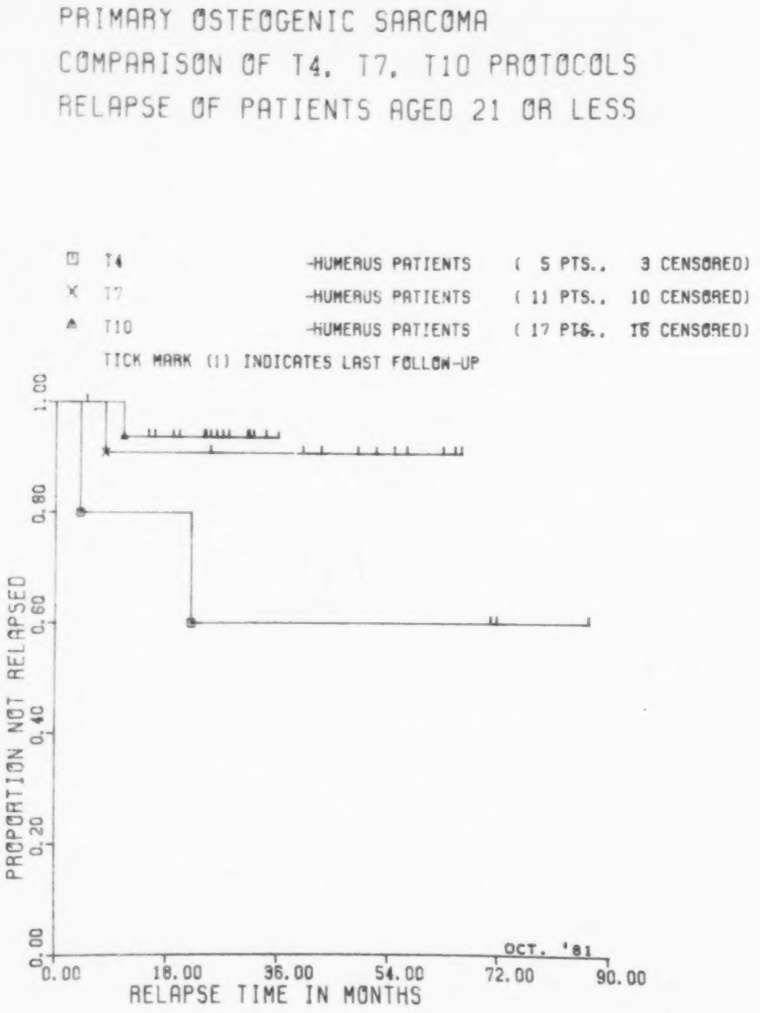

FIGURE 7.—Kaplan-Meier plots of continuous DFS of patients with primary osteogenic sarcoma of the humerus. Because of the small number of patients treated with the T-4 protocol and the very high continuous DFS rates, there is no statistically significant difference among patients treated with the 3 different protocols.

on the price of the 50-mg vial of methotrexate multiplied by 300 to arrive at the expected price of a 15-g dose of methotrexate. This price is artificially high and unwarranted. The actual cost of methotrexate is probably about \$10/g, the price that the National Institutes of Health, Division of Cancer Treatment, pays for the high-dose formulation of methotrexate. With wider acceptance of the treatment and the definition of its role in other diseases, the wider demand for methotrexate would probably lower the price that is currently being charged by commercial sources. The current cost to the National Institutes of Health per treatment is ≤ \$300, about the cost of a dose of cisplatin (120 mg/m²). In addition, the cost for a patient bed-day at our hospital is > \$450.00. The use of the outpatient department to administer treatment to our patients with osteogenic sarcoma has in effect made the actual cost of their treatment much less than that for patients with other malignancies requiring hospitalization.

Finally, effective chemotherapy for osteogenic sarcoma is difficult to perform and probably should not be performed by those inexperienced in the use of high-dose methotrexate with leucovorin rescue or by those physicians at institutions where fewer than ten patients per year will be treated. It would require at least this many patients to justify the work involved in monitoring patients following treatment and to give the responsible investigator sufficient experience to safely and effectively treat these patients. In view of the national incidence of osteogenic sarcoma and the number of designated cancer centers, the above consideration would limit the treatment of osteogenic sarcoma to only the larger designated cancer treatment centers. As can be seen from the data presented above, we treated > 50 patients and evaluated the effect of treatment prior to understanding the safe and effective use of high-dose methotrexate. It is unwise for oncologists who will treat fewer than ten patients per year to be involved in the treatment of osteogenic sarcoma. The majority will use protocols that they have little experience with and will have to "process" the patient through the protocol flow sheet. Although the recipe may be the same, the results can be expected to be different.

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Is It Ethical Not To Conduct a Prospectively Controlled Trial of Adjuvant Chemotherapy in Osteosarcoma?[1]

Beverly Lange[2,*] and Arthur S. Levine[3]

The medical literature contains only one report of a randomized, prospectively controlled study comparing surgery alone to surgery plus adjuvant chemotherapy in the treatment of osteosarcoma: the Mayo Clinic study (1). The estimated 2-year survival in this study is 74%, and the estimated 2-year disease-free survival is 52%. No statistically significant differences in survival or disease-free survival exist between the group receiving adjuvant chemotherapy and that receiving surgery alone. The sample size is small, 37 patients at the time of report; the followup is short, a median of 2 years in 1981; and the data have not yet been subjected to peer review (the report mentioned is preliminary, having appeared as a letter to the editor). Nonetheless, this randomized controlled study is the most powerful tool available to date to assess the relative benefits of surgery and chemotherapy in the treatment of osteosarcoma. Its results support two impressions abstracted from many earlier historically controlled or uncontrolled studies: *(a)* that a patient with osteosarcoma diagnosed in the past decade has a better chance of surviving for at least 5 years than a patient with the disease diagnosed earlier in this century and *(b)* that it is not necessarily correct to assume that chemotherapy is responsible for the improvement in survival (2–7). The trend toward improved survival and substantive reservations about the role of chemotherapy have led a number of other institutions to consider a prospectively controlled study comparing surgery alone to surgery plus adjuvant chemotherapy in the primary treatment of osteosarcoma. The purpose of this presentation is to justify such a study in 1982.

Except for the current Mayo Clinic study, the evidence for any improvement in overall survival, unrelated to the use of adjuvant chemotherapy, is largely circumstantial. The Southwest Oncology Group (SWOG) began clinical trials of adjuvant therapy in 1963. Although no improvement in continuous relapse-free survival was seen in the periods 1948–1962, 1962–1968, and 1969–1974, 2-year survival improved from 23% to 40% and 41% during the three respective periods (6,8,9). Presumably, better retrieval of patients who developed metastases accounts for the improvement (9). In 1971, Martini et al (10) documented that resection of pulmonary metastases could greatly prolong the survival of, and possibly cure, as many as 27% of patients with metastatic disease, and Rosenberg et al (11) have shown that 61% of patients with pulmonary metastases could be rendered disease-free by surgical resection. The experience of the Mayo Clinic paralleled that of SWOG in that 2-year survival increased from about 20% before 1968 to 50% between 1969 and 1971. In contrast to the SWOG experience, continuous relapse-free survival at the Mayo Clinic improved from 13% to 20% before 1969 to 42% in 1971 and has continued to improve throughout the decade. Again, while improvement in overall survival can be attributed to the surgical removal of metastases, the evidence suggests that other variables are responsible for the apparent improvement in continuous relapse-free survival (2). The Swedish study of leukocyte interferon as an adjuvant in the treatment of osteosarcoma (12) is a case in point: in 1977, continuous disease-free survival in an interferon-treated group was 50% at 2 years, but disease-free survival in a concurrent but nonrandomized control group was 35% rather than < 20% as anticipated. More recently, a Norwegian national study (13) has shown that overall survival in patients with osteosarcoma improved from 23% prior to 1975 to 47% after 1975 when adjuvant chemotherapy trials began in that country. Although the Norwegian data demonstrate that the introduction of adjuvant chemotherapy coincided with improved survival, the authors comment that "early amputation, intensified clinical followup, and surgery for metastases may have also contributed to overall survival." All of the studies cited and many others suggest that the impact of adjuvant trials may, in part, have been to render this disease treatable in the minds of physicians who directed patients to definitive surgical attention at an earlier point in time than had been the case historically.

The relatively recent and unexpected improvement in both continuous disease-free survival and overall survival, conceivably independent of, or only indirectly related to, adjuvant chemotherapy, contrasts sharply with all previous experience with this tumor. The natural history of osteosarcoma prior to the widespread use of

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[1] Received Apr 5, 1982; accepted Apr 8, 1982.

[2] Division of Oncology, The Children's Hospital of Philadelphia, PA.

[3] Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Beverly Lange, MD, Oncology Clinic, The Children's Hospital of Philadelphia, 34th and Civic Center Blvd, Philadelphia, PA 19104.

adjuvant chemotherapy has been reviewed extensively (2-7,14-16). Survival rates were consistently poor here and abroad. Among patients with nonmetastatic osteosarcoma treated with amputation or palliative irradiation, 50% developed metastases 6-10 months from diagnosis, 70%-80% had metastases at 12 months, and only 20% survived for 2 years (1-18). Based on the reproducibility of these numbers from institution to institution and from decade to decade, investigators in the late 1960s and early 1970s understandably concluded that historical controls provided a reliable and valid means of measuring the impact of adjuvant therapy on survival. Needless to say, for historical controls to be a useful statistical instrument, the historical group must be precisely comparable to the current study group. The unexpected improvement rate in 2-year survival to ≥ 40% in patients with osteosarcoma has therefore raised the obvious questions: Are historical and contemporary groups really comparable? And if they are not comparable, why not? Has the natural history of osteosarcoma changed? Has tumor histology or other aspects of biology changed? Have diagnostic maneuvers, surgical intervention, supportive management, or the timing of these events changed? What is the hard evidence that any given chemotherapeutic agent or regimen is effective, either in the treatment of overt disease (primary and metastatic) or in the adjuvant setting?

It is difficult to explain a change in prognosis by a change in natural history. Although theoretically the natural history of a tumor might change, there is no evidence that any form of human cancer has actually changed its biologic behavior. The incidence may change (lung cancer in women) or the host may change (Kaposi's sarcoma as seen in the current epidemic among young male homosexuals), but natural history as determined by biologic behavior seems stable. Factors that may be interpreted as alterations in the natural history of a tumor are more likely indirect manifestations of changes in selection and/or management.

Biologic variables in osteosarcoma surely exist, producing known and unknown prognostic factors, but they are not easily demonstrated and have not even been addressed in most adjuvant studies. The one prognostic variable that most investigators have agreed on historically is site; more distal lesions have been associated with an improved prognosis (7). Tumor size appeared to be an important variable before the chemotherapy era: patients with smaller tumors fared better with surgery alone than patients with large tumors (17). Few adjuvant studies using historical controls have compared mean tumor size. However, a National Cancer Institute (NCI) study showed that a reduction in tumor size was, in fact, apparent: the mean product of diameters of tumors on anteroposterior radiographs was 113 cm^2 in historical controls and 65.9 cm^2 in an adjuvant trial conducted after 1975 (18). This difference may represent a trend toward earlier diagnosis. At any rate, it suggests that current and historical groups may not be comparable with regard to prognostic variables.

The duration of symptoms prior to diagnosis has been cited as another variable, but it is a less objective parameter than tumor site or size, and perhaps it is more subject to changes in diagnostic trends. In the past, a relatively long lag time between the onset of symptoms and diagnosis was considered favorable: it suggested a relatively indolent lesion (7,17). Today, a change in attitude from pessimism to optimism may well bring all patients to an earlier definitive diagnosis. If the assumption that earlier diagnosis is responsible for improved survival is correct, a long lag time will not become unfavorable. Thus, retrospective analysis of apparent changes in the duration of symptoms is likely to be unrewarding and confusing.

Use of the number of patients with overt pulmonary metastases at diagnosis as a method to compare current patients receiving adjuvant therapy with historical controls is complicated by similar confounding variables: increased thoroughness in the search for metastatic disease at diagnosis by whole-lung tomography and computerized axial tomography will eliminate many more patients from the localized-disease category than had been true historically. Retrospective analysis (18-21) has shown, not only that tomograms reveal more lesions, but that the number of patients who would have been diagnosed as having metastatic disease only because of the increased sensitivity of computerized tomography (CT) scanning ranges up to 20%. The use of routine radionuclide bone scanning can similarly alter the composition of the population under study. These variables may play a significant role in changing apparent survival rates; they may narrow the window of patients selected for adjuvant treatment.

Some of the early studies of adjuvant chemotherapy or immunotherapy (eg, interferon) included patients with the less malignant variants of osteosarcoma, parosteal sarcoma, periosteal osteosarcoma, "low-grade intraosseous" osteosarcoma, and chondrosarcoma (16,22). Moreover, not all studies have employed a uniform grading system past and present. At the National Institutes of Health, fewer tumors with histologically high grade (III and IV) were diagnosed after 1975, but this variable alone could not account for the improvement in survival (18). The point is well-taken that histology should be considered as a variable prospectively and that biopsy and surgical specimens should be reviewed by a pathologist who is experienced in the interpretation of malignant bone tumors (23). However, when favorable histologic variants are eliminated from analysis by expert pathologists, survival of patients with osteosarcoma in the past remains at 20% and in the present, ≥ 40%.

Recently, more publicity has attended chemotherapy than surgery in the treatment of osteosarcoma; however, significant changes in surgical techniques have also taken place in the past decade. As noted above, the innovation that has had the greatest impact on overall survival is the resection, and repeated resections where necessary, of pulmonary metastases. Investigators at the Mayo Clinic, Memorial Hospital, NCI, and elsewhere have demonstrated that many patients who develop pulmonary metastases can be rendered disease-free for years by aggressive removal of their lesions (2,8,10,11). Other changes include the greater use of transmedullary amputation and various limb salvaging procedures. The development of limb salvaging procedures may indirectly have influenced the duration of continuous disease-free survival by reducing the anxiety associated with the prospect of amputation, thereby expediting early referrals of patients to cancer centers, ie, setting the clock that times the interval until metastases are detected at an earlier hour.

Other variations in surgical techniques need to be addressed when surgery alone (historically and currently) is compared to surgery plus adjuvant chemotherapy. Surgeons at the Mayo Clinic, which has the best results with surgery alone to date, do not use a proximal tourniquet at biopsy; they evaluate the biopsy material by frozen section and proceed directly with definitive ablative surgery (24). Most institutions do not have pathologists who are experienced in interpretation of malignant bone tumors or surgeons prepared to amputate on the basis of a frozen section. It is possible that no other institutions will be able to reproduce the Mayo Clinic's success with surgery alone unless they too eliminate the delay of days to weeks sometimes occasioned by two-stage operations.

At this point, no single variable has emerged to explain the differences in survival between historical controls and current patients. It may well be that the combination of all of the factors noted above has contributed to both an apparent and real improvement in survival. However, the improvement in survival has coincided with the introduction of organized trials of adjuvant chemotherapy. The selection of agents for use in these trials was based on phase I and II studies (7,25) of chemotherapeutic agents and combinations in patients with measurable disease; these studies have unequivocally demonstrated that some agents are tumoristatic and infrequently tumoricidal in a therapeutic setting. Some early studies made no distinction between stabilization of disease, partial response, mixed responses, and objective versus subjective responses. Moreover, some response data are confounded by intercurrent exposure of the patient to radiotherapy and/or surgery. The list of agents or combinations that have yielded objective tumor regression in more than one or two patients in any series is short, and the number of patients reported is small. The following agents and combinations have demonstrated activity: doxorubicin and cisplatin as single agents; high-dose methotrexate and leucovorin calcium (with or without vincristine); the combination of bleomycin, cyclophosphamide, and dactinomycin (BCD) (26); and in adults, doxorubicin and dacarbazine. Only the efficacy of doxorubicin (27,28) and high-dose methotrexate (29,30) have been confirmed at more than one institution. Although early studies documented response rates of 40%–80%, recent, more conservative estimates suggest that objective tumor regression occurs in 10%–20% (with the highest response, 37%, reported for BCD) (26–31). Moreover, in most studies, complete responses to any drug or combination have been rare, comprising < 10% of all responsive cases (7). These studies in patients with overt disease provide the only clues as to which of these drugs and combinations might benefit patients when given as adjuvant therapy and also suggest that the impact of adjuvant therapy might be modest at best.

In many adjuvant trials, doses and schedules of the agents employed differ from those used earlier in treating overt disease; they also differ from trial to trial. While the three successive adjuvant trials at the Sidney Farber Cancer Institute have suggested a dose-response effect with high-dose methotrexate (32), the Children's Cancer Study Group (in CCSG study 741) has failed to demonstrate any advantage of high-dose methotrexate over moderate-dose methotrexate (I. Ertel, personal communication). It had been thought that serum levels of methotrexate in the 10^{-4} M range were necessary to penetrate into osteosarcoma (levels not achieved with moderate-dose methotrexate), but the CCSG and others report a survival rate > 40% in patients treated with lower concentrations. Furthermore, > 40% of patients treated with adjuvant combinations previously judged as inactive or untested as single agents (ie, vincristine, bleomycin, dactinomycin, low-dose methotrexate, melphalan, and prophylactic pulmonary irradiation) now survive (7). More than 40% of patients treated with noncytotoxic agents of highly questionable effectiveness in this disease also survive; these agents include transfer factor (33), tumor cell vaccine (34), warfarin (Coumadin) (35), and interferon (12). Compared to the widely quoted series of historical controls, virtually all adjuvant agents have yielded statistically significant improvements in survival.

The very diversity of agents and techniques credited with improving disease-free survival in this notoriously resistant tumor led Carter and Friedman (5) to observe that "with more careful case selection combined with earlier data analysis, almost anything can seem to be positive when compared to the earlier data base." It is also curious that in most studies, the combination of active drugs (eg, high-dose methotrexate with or without doxorubicin) did not improve results, unlike the situation with most other responsive tumors. These results suggest either that no adjuvant treatment is effective or that all are equally effective; ie, no more cells are killed even though more drugs with different intracellular targets (and different mechanisms of resistance) are added. Finally, while the initial life-table analyses in the first adjuvant reports projected relapse-free survival rates > 80% (8,16,36), actual 2-year, relapse-free survival rates now range from 26% to 65% (6,7), not dissimilar to disease-free survival in contemporary control subjects treated with surgery alone or with presumably ineffective therapy.

Thus, > 40% of patients treated with adjuvant agents that are generally accepted as active in overt osteosarcoma (ie, methotrexate, doxorubicin, and cisplatin) now survive, continuously free of disease (7). When the results of various adjuvant regimens are compared, the differences usually fail to reach statistical significance because of wide ranges in confidence limits (the sample size in most trials is small) and because of the relatively narrow range of results. Two exceptions are results in patients who were treated according to the T-7 or T-10 protocol at Memorial Hospital, where continuous relapse-free survival at a median of 2 years is > 90% (37,38), and in patients at the Sidney Farber Cancer Institute who were treated according to the third consecutive adjuvant protocol (32). These exceptions provoke the question of the role of the investigator or institution in influencing outcome. Investigators at these two institutions have been pioneers in the treatment of osteosarcoma; their experience is more extensive, and possibly their commitment to detail is more intense, than in institutions that are trying to reproduce their results. It is not unusual that the institution that has developed a technique remains the most adroit and successful in its application. However, it is also not unusual for second-generation studies to demonstrate that an untreated or conventionally treated control group has better results than anticipated (39).

If we accept as a given that a highly predictable 40%–50% of all osteosarcoma patients treated with a diversity of adjuvant agents and regimens now survive continuously free of disease for ≥ 2 years, there remain the questions of why Rosen et al (37), using an approach that does not differ radically from that of others, report a figure for continuous disease-free survival > 90%. Certainly, extensive experience and commitment to detail may account for some of the difference. However, it is difficult to conceive that increasing the dose of methotrexate to > 8 g/m^2 in young children—the point that Rosen emphasizes—accounts for this apparently striking improvement. Only a minority of the osteosarcoma population is sufficiently young (ie, remote from achieving their full adult stature) that this point is germane, and the results in this limited group should therefore not have great impact on the overall results. Moreover, the CCSG study, as discussed above, fails to reveal any dose-response relationship within the range employed by that group. Alternative explanations for this striking difference can be entertained: Rosen (38) states that the number of patients who are eligible for limb salvage operations at his institution has been falling, ie, patients are now being referred with more advanced lesions, beyond the point of resectibility. If such patients still are free of metastases on referral to Memorial Hospital, as determined by sensitive CT scanning, they must surely have a biologically favorable disease, possibly with a reduced propensity for early metastatic spread.

Because of coincidence in the use of adjuvant chemotherapy and improvement in survival in osteosarcoma and because of the proven efficacy (however slight) of some agents in patients with measurable disease, adjuvant chemotherapy continues to be used in an uncontrolled fashion in most cancer centers throughout the world. However, the apparent and unexpected improvement in continuous disease-free survival since 1970, in a small number of patients treated only surgically, has so confounded the interpretation of results of adjuvant chemotherapy trials that a growing number of investigators, in addition to those at the Mayo Clinic, have come to believe that they are ethically and intellectually obliged to conduct a randomized, prospectively controlled study comparing surgery alone to surgery plus adjuvant chemotherapy. When, in the mid-1970s, reservations about a cause and effect relationship between adjuvant chemotherapy and survival were first raised and when prospectively controlled studies were proposed, such trials were considered to be nihilistic and frankly unethical. The cost, the toxicity, and the psychosocial disruption imposed by adjuvant chemotherapy regimens were thought to be far outweighed by the benefits that then appeared newly available to all patients with osteosarcoma. Furthermore, the tremendous publicity afforded this apparent success made any controlled study difficult to undertake because of the demands for chemotherapy made by referring physicians and patients. Thus, in 1982, after scores of clinical trials, hundreds of abstracts and manuscripts, and extensive agonizing over what constitutes a responsible approach, we still do not know if adjuvant chemotherapy improves continuous disease-free survival or overall survival in most patients with osteosarcoma. It seems evident that osteosarcoma is a relatively unresponsive tumor with respect to the majority experience using currently available chemotherapy and radiotherapy and that much of the enthusiasm for chemotherapy in this disease, whether as an adjuvant to resection of the primary tumor or to metastatectomy, has been based on results that may reflect subtle distortions in case selection, assessment, and prognostic factors, as well as the possibility of observer bias and insufficient followup in such an enthusiastic climate. Moreover, interpretation of long-term survival (as opposed to cure) is presently complicated by the frequent failure to distinguish patients with continuous disease-free status after one thoracotomy from patients who have had multiple sequential thoracotomies, including very recent ones.

If chemotherapy were innocuous, one might give the results of adjuvant chemotherapy the benefit of the doubt. However, the long-term effects of present regimens have not been completely evaluated. Recently at the NCI, cardiac abnormalities have been demonstrated by gated-ECG radionuclide scanning in 50% of young adults treated with doxorubicin as an adjuvant to surgical resection of soft tissue sarcomas, and congestive heart failure has occurred in 16% of such patients. This is but one possible morbid sequel of the regimens currently used and serves to emphasize the necessity that we be convinced at a statistically significant level of the ultimate risk-to-benefit ratio of these treatments. For this reason, investigators of the Pediatric Oncology Group, the National Cancer Institute, the Sidney Farber Cancer Institute, Stanford University, St. Jude Hospital, M. D. Anderson Hospital, the Children's Hospital of Philadelphia, and other institutions are now beginning a multi-institution, prospectively controlled (surgery alone), randomized clinical trial of a regimen approximating that reported to have yielded the best results to date (the T-10 protocol described by Rosen [38]). This study appears to be long overdue. It is certainly true that a large multi-institution study may not yield as good a result as Rosen's single-institution trials, but analysis of the results and quality of participation by center will address this point.

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The Development of New Cancer Drugs: A Joint Venture in Public Policy by the National Cancer Institute and Food and Drug Administration[1,2]

J. Richard Crout[3,*]

I am delighted and honored to participate in your program this afternoon. It is a mark of Dr. John Ultmann's generosity as president of the American Society of Clinical Oncology, and of his sensitivity to public affairs, that I am here. Last fall we both appeared at a Congressional hearing, where I discussed a series of innovative policy agreements that have been made in recent years between the National Cancer Institute (NCI) and the Food and Drug Administration (FDA) to foster the development of cancer chemotherapeutic agents. I was of course very pleased when he called some time later to ask if I would share his presidential address time at this meeting to report to you on this same topic. I cannot imagine a warmer gesture or a better forum, and I am very grateful to him.

Let me begin by reminding you, in broad terms, how new cancer drugs are developed and distributed in this country. Most proposed new cancer chemotherapeutic agents, even those originally discovered by drug firms, funnel through the NCI evaluation program. Under this program many new molecules are tested for antineoplastic activity in animals, and a few are selected for subsequent toxicologic testing in animals. From this group are selected the compounds for evaluation in phase I and II clinical trials. If a compound appears to be effective in humans on the basis of remission rates for one or more tumor types, it is evaluated more broadly in phase III trials conducted by cooperative groups and individual investigators. If the drug is especially promising for a particular tumor type by comparison with other available therapy, a demand for use of the drug in treatment develops at this point, even though it is not yet marketed. It has long been NCI and FDA policy to permit so-called treatment or compassionate distribution of an investigational drug to cancer patients under these circumstances. Later still in the developmental process, a drug firm will be licensed by the NCI to combine the animal and clinical data on the drug and submit this information to the FDA in an application called a new drug application (NDA). After review and approval of this NDA, the drug is marketed by a manufacturer in the usual manner for all drugs.

The point I would emphasize is that all of the drugs distributed by the NCI are in one stage or another of the investigational process and are investigational under the law. This means that all such distribution must be for research or treatment under an investigational new drug application (IND) submitted to the FDA. Such use thus requires research protocols, written informed consent, institutional review board (IRB) review, and the mandatory reporting of serious adverse reactions; these are all requirements for investigational drugs but not for marketed drugs.

Against this background, let me comment briefly on certain problems that developed between the NCI and the FDA in the mid-1970s. For a variety of reasons that seemed to come together at about the same time, both institutions came to realize that they needed to do a better job of managing the whole process for investigational cancer drugs. Also, tension developed between the institutions over such matters as the quality of applications, late annual reports, and our respective roles. The situation came to a head when the FDA turned down several INDs, some for procedural reasons and some for scientific disagreement over the safety of proposed studies. You are familiar with the outcry against the FDA regulation by some members of the cancer community at that time.

It was in that environment that Dr. Vincent DeVita (then director of the Division of Cancer Treatment, NCI) and I, along with our staffs, began to undertake the joint ventures that are the central theme of this talk.

First, we developed a memorandum of understanding signed by the Director of the National Institutes of Health and the Commissioner of Food and Drugs outlining the respective roles of the two institutions. NCI reaffirmed its policy of following all FDA regulations with respect to investigational new drugs. FDA agreed to accept from NCI a master file detailing its internal procedures and providing general information applicable to all NCI applications, thereby reducing the need for redundant submission of paperwork. FDA also agreed not to turn down INDs from NCI on procedural or paperwork grounds. Instead we would use the master file as the focus for discussion for procedural problems and individual INDs as the focus for discussion of scientific issues on specific drugs. We also agreed as a matter of internal policy to permit NCI to correct deficiencies in

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[1] Received May 18, 1982; accepted June 8, 1982.

[2] Delivered at the National Meeting of the American Society of Clinical Oncology, St. Louis, MO, Apr 26, 1982.

[3] Bureau of Drugs, Food and Drug Administration, Rockville, MD.

[*] *Reprint requests to:* J. Richard Crout, MD, Office for Medical Applications of Research, National Institutes of Health, Bldg 1, Rm 216, Bethesda, MD 20205.

its INDs without delaying clinical trials unless a deficiency posed a clear safety issue. NCI in turn increased its staff to improve the quality of its INDs. The result of all these changes has been very positive: a smooth IND process for the past several years, with no significant delays in processing INDs submitted by the NCI.

Second, NCI carefully drafted, and FDA carefully reviewed, the master file describing the NCI drug development and distribution system. The result has been a far greater understanding of the other institution by both staff and management of NCI and FDA.

Third, NCI greatly revamped its drug distribution system by classifying its investigational drugs into three categories known as A, B, and C. Group A drugs correspond roughly to those in phase I and early phase II, while group B drugs are those in late phase II and phase III of the investigational process. Group C drugs are those investigational drugs with sufficient evidence of particular value in the treatment of a tumor type that they are made available to oncologists for patients with those types of cancer. The group C category thus provides a legal mechanism for investigational drugs of special promise to be made available for the treatment of patients. Before a drug goes into group C, NCI supplies the available information to FDA and the data are reviewed by our Oncological Drugs Advisory Committee. The committee's reviews are thoughtful and rigorous. They commonly occupy several hours of a meeting, and although uncommon, turndowns on grounds that the evidence is premature have occurred.

Fourth, NCI proposed a modified set of guidelines for toxicologic studies of new cytotoxic drugs prior to their evaluation in humans. The new guidelines eliminate testing in the monkey, decrease the studies conducted in dogs, and increase testing in mice. These new guidelines have proven to be the most controversial of the policy changes adopted in recent years and, to some extent, are still evolving with experience. Nevertheless, they have been reviewed extensively by FDA, NCI, Public Health Service, and several outside advisory groups, and I expect the basic approach in these guidelines to remain unchanged so long as our experience remains favorable. I would emphasize to this audience that those of you who conduct clinical trials of cancer drugs have a critically important role in determining the success of this new toxicologic approach. The primary intent of animal testing under the new guidelines is to estimate a safe initial dose for the beginning of phase I trials. It is not to establish the organ toxicity of the drug, the usual objective in most toxicologic work. The strategy adopted by the guidelines is that, regardless of the findings in animals, potential toxicity to any organ must be presumed for this highly toxic class of drugs and be specifically sought in humans. The burden for identifying and limiting organ toxicity in patients has, of course, always rested with those who study these agents in clinical trials and use them in the care of patients. The strategy followed in the new guidelines makes that burden explicit.

Fifth, representatives of FDA and NCI, at both staff and management levels, began to meet periodically to discuss problems of mutual concern. These meetings have increased understanding and have served to identify problems at an early stage.

I feel confident today that the major problems of the past are solved as a result of the policy steps taken jointly by the FDA and NCI. Several INDs for new chemotherapeutic agents have been received from the NCI and approved in the past year. The future looks bright and busy for new drug development in the cancer area.

However, all controversy has not disappeared, nor has life suddenly become dull at either the FDA or the NCI. There will continue to be occasional scientific disagreements in interpreting the data on individual drugs. The two institutions have different roles and, like authors and editors or athletes and coaches, are inextricably bound in a common enterprise. But the product of that enterprise, extracted though it may be with some Sturm und Drang, is better than either could produce alone.

Because people are human, there will also continue to be differences over the policies applied to new drug development and regulation. For every person who believes that the regulation of cancer drugs is too lax, there is someone else who thinks that the FDA should not regulate such drugs at all. For everyone who feels that the new toxicologic guidelines are too sparse, there is someone else who wants to extend them to other classes of drugs. For those who believe in the particular value of randomized controlled trials, there is someone else to argue the value of historical trials. These controversies are likely to continue indefinitely. If one of them erupts now and then in the press or a Congressional hearing, it is not to be taken as a sign that something is wrong with the regulatory process. Neither does it mean that every voice represents an institution or is announcing new policy. Such eruptions may simply be a sign that intelligence and thoughtful debate are still alive in Washington—something I gather you wonder about from time to time.

I would like to take the opportunity to pose three challenges to your specialty; all are narrowly focused and relate to the development and regulation of cancer drugs. The first challenge concerns the quality of informed consent by patients who participate in cancer trials, particularly phase I studies. While the articles in *The Washington Post* last fall have been widely recognized as inaccurate and unbalanced, there was running through them one seemingly credible theme, namely that at least some patients do not have a full picture of the potential risks and benefits of phase I studies. The same theme was echoed in the related Congressional hearings. I do not presume to know where righteousness lies on this complex issue. However, I do believe that I can observe an undercurrent of concern in the Washington press and in the Congress over whether all patients in phase I studies are being approached forthrightly and honestly. My observation is that those of you who face the problem of helping patients with difficult choices at the end of their lives are perceived, whether deserved or not, to have both a challenge and an opportunity: a challenge to assure balance in the informed consent process and an opportunity to win greater public trust in the early drug development process.

A second challenge I would pose to you is to maintain a high sensitivity to the potential for discovering new serious adverse reactions to cancer drugs. History tells us that such effects, particularly those that are delayed or cumulative, are discovered almost entirely by astute clinicians who are prepared to see the new and novel. I would urge each of you to be alert to the contribution to drug safety that you can make through perceptive observation.

Finally, I believe we have a common challenge: to improve the regulation of all drugs, including cancer drugs. I recognize that it is popular to think of regulation as something that occurs in Washington apart from the rest of the country. I recognize also that the escalation of regulatory requirements or procedural intricacies is commonly attributed to empire building by bureaucrats. I would not deny that there is some truth in both statements, but I would caution against accepting these popular dicta too easily. The real problem, as I see it, is a phenomenon known as "regulatory creep," which occurs for the same reasons that technologic advance or procedural complexity occur in other fields. Regulatory creep occurs because good people who are intelligent and well-motivated want to do a better job. A toxicologist wants to add a new test because it is technically possible and potentially helpful. A data-processing expert wants to add a new form to make his or her work more efficient. A dean wants to add another committee review in the interest of winning broader consensus in the institution. An investigator wants to add additional laboratory tests to a clinical trial as part of a personal research interest. And so it is that we gradually make the simple complex and the straightforward costly.

The solution is not simply to blame the regulators. The solution is to work together to find the many small ways in which we can each contribute to the reversal of regulatory creep—through greater self-restraint, by building trust, by keeping review procedures to a minimum, and by encouraging innovation and freedom as well as accountability in the conduct of clinical research. The FDA is working hard to revise its regulations governing the investigational drug process, with these principles in mind. While it will be some time before a revised version of these regulations can be published, I believe the agency will ultimately improve the process without compromising the basic essentials of our drug regulatory system. To the extent that you too can limit procedural and technical requirements in committee reviews in your own hospitals and medical schools, we will together make progress in the search for simplicity.

In closing, I thank Dr. Ultmann again for the privilege of joining your program. The combined efforts of our nation's biomedical research program, our drug and medical device industries, and your specialty of medicine are now paying off in the ever-improving survival of many cancer patients. That is an enormous medical achievement. We at the FDA share your pride in accomplishment and your hopes for the future.

Vindesine: An Effective Agent in the Treatment of Non-Small Cell Lung Cancer[1,2]

Becky E. Furnas, Stephen D. Williams, Lawrence H. Einhorn,* and Melody A. Cobleigh[3]

A dose of 3–4 mg/m^2 of vindesine (DVA) was given by iv push weekly to 33 patients with non-small cell lung cancer. Twenty-nine patients were evaluable for response. All evaluable patients had a performance status > 50% and measurable disease, and received at least two consecutive weekly doses of DVA. Standard response criteria were used. Among 15 patients with squamous cell carcinoma there were four partial responses and one complete response; three of eight patients with adenocarcinoma had partial responses. The overall response rate was 28% (eight responses among 29 patients). Myelosuppression was moderate, but two patients were hospitalized for granulocytopenic infections. The most common dose-limiting side effects were due to neurotoxicity. Four responding patients had treatment stopped because of neurotoxic reactions. DVA appears to be among the most active drugs in non-small cell lung cancer, but improved dose schedules that reduce neurotoxicity would be highly desirable. [Cancer Treat Rep 66:1709–1711, 1982]

Vindesine (DVA) is a semisynthetic vinca alkaloid derived from vinblastine (1). Phase I studies by Dyke and Nelson (1) recommended that DVA be given at a dose of 3–4 mg/m^2/week. In early laboratory trials it appeared that neurologic toxicity with DVA would be less than that with previously tested vinca alkaloids (2).

A partial remission in one of four patients with squamous cell lung cancer was first reported by Bodey and Freireich in 1976 (3). Table 1 summarizes available single-agent data for DVA in patients with non-small cell lung cancer (NSCLC). Accordingly, DVA was evaluated in carefully selected patients with NSCLC.

PATIENTS AND METHODS

From September 1978 to February 1980, 33 patients with NSCLC were treated with DVA. There were 32 males and one female, with a median age of 58 years (range, 37–81). All patients had a pathologically confirmed diagnosis. The patient population is described in table 2.

Entry in the study required normal hematologic function, a Karnofsky performance status ≥ 50%, and measurable disease on a standard posteroanterior and lateral chest X-ray and/or physical examination. All patients were fully recovered from the toxicity of prior treatment. Baseline liver and renal function was assessed. Bone, liver, and brain scans were obtained as indicated. Informed consent was obtained from all patients.

DVA was given at a dose of 3 mg/m^2/week by iv push. Doses were escalated by 0.5 mg/m^2 every fourth week to a maximum of 4 mg/m^2 if no significant toxic reactions occurred. Treatment was delayed 1 week and/or a 25% dose reduction was made for severe hematologic toxic effects (platelet count < 50,000/mm^3 or wbc count < 2000/mm^3). Neuromuscular toxicity could also necessitate a 25% dose reduction or a change from a weekly to an every-other-week schedule.

A complete remission (CR) was defined as the disappearance of all signs and symptoms of disease. A partial remission (PR) was defined as a ≥ 50% reduction in the sum of the products of perpendicular diameters

TABLE 1.—Results of DVA therapy in NSCLC

Authors (ref No.)	No. of patients	No. of responses (%)
Gralla et al (4)	46	10 (22)
Mattson et al (5)	37	4 (11)
Grundtvig et al (6)	34	8 (24)
Furnas et al (present study)	29	8 (28)

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[1] Received Nov 19, 1980; revised June 9, 1982; accepted June 16, 1982.

[2] Supported in part by Public Health Service grant CA-19657 (Southeastern Cancer Study Group) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Eli Lilly and Company. Dr. Einhorn is an American Cancer Society Clinical Professor of Oncology.

[3] Department of Medicine, Indiana University Medical Center, and the Indianapolis Veterans Administration Medical Center.

**Reprint requests to:* Lawrence H. Einhorn, MD, Indiana University Medical Center, University Hospital, Rm 582-N, 1100 W Michigan St, Indianapolis, IN 46223.

TABLE 2.—Response to DVA therapy

	No. of patients	No. of responses
Cell type		
Squamous cell carcinoma	15	1 CR, 4 PRs (33%)
Adenocarcinoma	8	3 PRs (38%)
Large cell carcinoma	3	0
Undifferentiated carcinoma	3	0
Karnofsky performance status		
80%–100%	22	6 (27%)
50%–70%	7	2 (29%)
Prior therapy		
None	16	3 PRs
Surgery	2	1 PR
Irradiation	7	1 CR, 2 PRs
Chemotherapy	3	0
Surgery + irradiation	1	0
Surgery + irradiation + chemotherapy	1	0
Irradiation + chemotherapy	2	0
Whole-brain irradiation + chemotherapy	1	1 PR
Prior vinca alkaloid therapy		
Yes	1	0
No	28	8

of measurable disease. All responses lasted a minimum of 4 weeks. Evaluable patients received at least two weekly doses of DVA.

Twenty-nine of the 33 patients were evaluable for response. Three patients refused further therapy after receiving one dose. A fourth patient had obstructive atelectasis with pneumonia and started receiving radiation therapy after two doses of DVA. Two of the 33 patients presented with brain metastases and received whole-brain radiation therapy as well as DVA.

Patients were followed with weekly cbcs and neurologic examinations. Tumor measurements as well as liver and renal function studies were repeated monthly.

RESULTS

Eight patients responded to DVA, as shown in table 2. Three of eight adenocarcinoma patients had PRs. Four PRs and one CR were achieved in 15 patients with squamous cell carcinoma; the CR was documented by whole-lung tomography. All eight responses were documented radiographically on chest X-ray, and one patient had a concomitant 75% decrease in a metastatic supraclavicular node as well. The overall response rate for the 29 evaluable patients was 28%. The median time to remission was 4.5 weeks (range, 1–8). The PRs lasted 4–18 weeks (median, 12); the CR lasted 26 weeks. The small number of patients in this study precludes drawing conclusions regarding the influence of disease site, cell type, performance score, or prior therapy on response. However, only one of seven patients who had received prior chemotherapy responded.

Two of the responding patients are alive at 8 and 15 months after DVA treatment. Three of the nonresponding patients remain alive at 13, 14, and 15 months after DVA. All patients currently have progressive disease. Median survival was 34 weeks for the responders and 24 weeks for the nonresponders.

Seven patients had from one to four doses of DVA omitted due to leukopenia (range, 800–2400 wbcs/mm^3). Two of the seven patients developed granulocytopenic infections that responded to antibiotics. On all but two occasions the wbc count had returned to acceptable levels within 1 week. Sustained myelosuppression was not seen.

Six patients had hemoglobin drops of 4.0–5.8 g/100 ml. None of the six patients required transfusion. Thrombocytopenia was not observed.

Alopecia developed in 15 of 22 patients evaluable for hair loss. The degree of alopecia appeared to be dose-related. Extravasation of DVA occurred on four occasions and resulted in a minor ulceration once.

Neurologic toxicity, the most frequent and dose-limiting side effect, was manifested by a variety of signs and symptoms. Peripheral neuropathy and generalized malaise occurred in 20 patients. Ten patients had scheduled dose increases delayed, doses decreased, or treatments changed to every other week due to neurotoxicity. Five patients (including four responders) who experienced difficulty walking or completing other motor skills ultimately had therapy terminated due to these toxic reactions. Moderate constipation occurred in five patients. One other patient was admitted to the hospital for a bowel obstruction. This did not resolve with conservative management, but at surgery the cause of obstruction was found to be adhesions. Blurred vision, jaw pain, and headache were occasionally reported.

Three patients developed fever and chills on the evening of their DVA injections. A severe generalized allergic rash developed in one patient 3 days after his first DVA injection, which appeared again upon rechallenge with the drug. This patient achieved a PR after these two doses but therapy had to be terminated.

DISCUSSION

The response rates of this disease-oriented phase II trial are comparable to those of Gralla et al (4) (22%) and Grundtvig et al (6) (24%) and are somewhat higher than that seen in the series of Mattson et al (5) (11%). Overall, by combining the results in all four series, responses have been seen in 30 of 146 (21%) evaluable patients (4–6). Thus, there has been considerable experience in disease-oriented trials with DVA and it is among the most active agents in NSCLC (7,8). If only patients without prior chemotherapy are considered, the cumulative response rate is 23% (26 responses among 115 patients) (4–6).[4]

In two studies (present series and ref 4) no difference was noted according to cell type, but overall, eight of 61 (13%) patients with squamous cell carcinoma responded versus four of 20 (20%) with large cell carcinoma and 18 of 62 (29%) with adenocarcinoma. It is difficult to make dogmatic conclusions from these data, but we feel there is no major difference in responsiveness among cell types.

In our experience, neurotoxicity has been a major problem. It is possible that lower doses, longer intervals, or different schedules [ie, continuous infusion (9)] may reduce toxicity. We currently utilize DVA at a weekly dose of 3 mg/m^2 for a maximum of 6 consecutive weeks and then give the same dose of DVA every other week; this has substantially reduced the neurotoxicity.

The relative merit of DVA as opposed to the old vinca alkaloids is unknown. A cumulative response rate of 11% for vinblastine (7) has been seen, and in two trials of vincristine, responses were seen in two of 37 (5%) and six of 35 (17%) patients (10). Of the three agents, it is likely that DVA is the most active.

We conclude that DVA has significant and reproducible activity in NSCLC, and combination chemotherapy and combined modality trials are warranted.

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Studies of Baker's Antifol, Methotrexate, and Razoxane in Advanced Gastric Cancer: A Gastrointestinal Tumor Study Group Report[1,2]

H. W. Bruckner,* J. J. Lokich, and D. M. Stablein[2]

In this multi-institutional study of advanced gastric cancer, 73 patients were evaluable for response or survival. Patients were treated with either triazinate (Baker's antifol), standard-dose methotrexate, or ICRF-159 (razoxane). Objective responses were seen in four patients receiving Baker's antifol, in three receiving methotrexate, and in none receiving razoxane. Baker's antifol produced a median survival of 18 weeks and methotrexate and razoxane produced a median survival of 8 and 9 weeks, respectively. Seventy of the 73 patients entered in this study had been previously treated, most frequently with combination chemotherapy regimens containing 5-FU or doxorubicin. This study appears to demonstrate that Baker's antifol is an active drug for patients with advanced gastric cancer, according to both response and survival criteria. Examination of the pretreatment prognostic characteristics of the patients further suggests that a possible survival advantage is due to treatment with Baker's antifol rather than the prognostic characteristics of the patients. [Cancer Treat Rep 66:1713–1717, 1982]

Advanced gastric cancer has shown response to several cytotoxic drugs, most notably, 5-FU, mitomycin, and doxorubicin (1–8). Response rates have been in the 15%–30% range. There have been suggestions in the literature that drug combinations involving 5-FU and either mitomycin, nitrosoureas, or cytarabine may increase objective response rates (1–3,6,7). Gastrointestinal Tumor Study Group (GITSG) investigations, however, did not show or suggest a survival advantage for any of these drug combinations (8,9). These phase II–III studies have found a survival advantage for 5-FU and semustine or mitomycin in combination with doxorubicin when compared to combinations without doxorubicin (8,9).

The GITSG was previously able to confirm (5) that doxorubicin was an active drug for patients with advanced gastric cancer, both as primary and secondary therapy. Secondary therapy produced three responses among 17 patients (8). The phase II study reported herein initially compared triazinate (Baker's antifol), standard-dose methotrexate, ICRF-159 (razoxzane), and high-dose methotrexate with leucovorin calcium in similar patients with measurable disease failing prior chemotherapy. The concept of directly testing the antifolate compounds was scientifically attractive, and razoxane was of additional interest due to its reported ability to prevent doxorubicin toxicity and tumor angiogenesis in preclinical systems (10). However, the high-dose methotrexate regimen was suspended because it proved impractical to accrue patients. It will not be considered in the remainder of this report.

METHODS

All patients selected for the study were required to have known gastric adenocarcinoma with histologic proof of unresectable, residual, recurrent, or metastatic disease. All patients were required to have measurable disease with an area of known tumor that could be clearly calibrated. Palpable hepatomegaly was acceptable if the liver was proven to contain metastasis and if a well-defined liver edge extended at least 5 cm below either the xiphoid process or the costal margin on quiet respiration. A positive liver scan was accepted if there was a clearly defined perfusion defect measuring at least 5 cm in diameter. Lesions visualized only by contrast roentgenography or lesions for which size would only be estimated, eg, pelvic or extrarectal masses, were not accepted. Contraindications to entry in the study included the following: recent surgery with resection

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[1] Received Jan 11, 1982; revised May 21, 1982; accepted June 7, 1982.

[2] Supported by contracts (CM) from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Participating institutions and investigators include: Mayo Clinic, Rochester, MN (*C. G. Moertel, M. J. O'Connell, J. Rubin, A. J. Schutt*, N01-CM-43783); Mount Sinai School of Medicine, New York, NY (*H. W. Bruckner, B. Kabakow, J. A. Storch*, N01-CM-53841 and N01-CM-07407); Roswell Park Memorial Institute, Buffalo, NY (*H. O. Douglass, Jr, H. R. Nava, R. A. Berjian, S. Milliron, P. M. Sweeney*, N01-CM-43782 and N01-CM-07410); Wayne State University, Detroit, MI (*L. Leichman*, N01-CM-07408); Yale University, New Haven, CT (*E. M. Livstone, J. Marsh, H. K. Wright*, (N01-CM-53843 and N01-CM-07339); New England Deaconess Hospital, Boston, MA (*J. J. Lokich*, N01-CM-43781); and The EMMES Corporation, Potomac, MD (*D. M. Stablein*, N01-CM-87193).

**Reprint requests to:* H. W. Bruckner, MD, Mount Sinai School of Medicine, Department of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029.

or bypass (< 3 weeks previously); recent abdominal exploration and biopsy only (< 2 weeks previously); recent radiation therapy or chemotherapy (< 4 weeks previously); total disability (Eastern Cooperative Oncology Group [ECOG] performance score of 4); severe malnutrition, severe nausea, or frequent vomiting; an active infectious process; and leukopenia (< 4000 cells/mm^3) or thrombocytopenia (< 150,000 cells/mm^3).

Patients were stratified according to initial performance status (ECOG scale of 0 or 1 vs 2 or 3); prior therapy with agents not used in the study vs prior razoxane vs prior antifolates; and serum bilirubin < 1.5 versus > 1.5 mg/100 ml. Patients were randomly assigned to the treatment arms, with the following exceptions: prior therapy with the study agent or its analog excluded patients from randomization to the arm containing the prior study agent; and patients with bilirubin > 1.5 mg/100 ml were not eligible for treatment with Baker's antifol.

Patients were required to have iv pyelograms and to have creatinine clearance > 70 ml/minute, serum creatinine < 1.5 mg/100 ml, or BUN < 25 mg/100 ml; they had to be ineligible for GITSG phase III combination therapy protocols either because of prior treatment with a study agent (5-FU, doxorubicin, or a long-acting alkylating agent) or because heart disease contraindicated doxorubicin. In practice, one institution did not perform creatinine clearance determinations. Patients were also considered to be ineligible if their caloric intake was < 1500 calories/day or if there was evidence of severe malnutrition, nausea or vomiting, or third-space fluid overload, eg, ascites, pleural effusion, or major edema. Patients were required to have provided informed consent in accordance with current institutional and federal regulations.

Baker's antifol was given as a 250-mg/m^2/day infusion over 1 hour for 3 consecutive days and was repeated every 3 weeks (Days 1–3, 22–24, etc). Standard-dose methotrexate was given as a 40-mg/m^2 bolus injection once weekly, and razoxane was given at a dose of 300 mg/m^2 three times a day orally for nine doses, every 4 weeks (Days 1–3, 29–32, etc).

For each regimen, weekly wbc and platelet counts were recommended, as well as blood chemistries and a comprehensive physical examination before each treatment course. Appropriate reductions in doses of subsequent courses were made if excessive toxicity was observed. For those patients with measurable disease, the criteria for objective response required all of the following: *(a)* reduction by at least 50% of the product of the longest perpendicular diameter of the most clearly measurable tumor lesion (if hepatomegaly was the indicator lesion, it was required that there be a reduction of the sum of measurements below the xiphoid and each costal margin by at least 30%); *(b)* no increase in the size of any other measurable lesion and no new lesions of malignant disease; and *(c)* no significant loss in body weight (> 5%) or deterioration of performance status (one level by ECOG score). The criteria for disease progression included: *(a)* an increase in the size of any measurable lesion by > 25%; *(b)* the appearance of new areas of malignant disease; and *(c)* a loss in body weight (> 5%) or the deterioration of performance status (one level by ECOG score).

RESULTS

The study was activated in September 1977. In September 1979, the group decided to keep each arm open until it accrued at least ten patients with an ECOG initial performance status of 0 or 1. In June 1980, accession to the razoxane arm was discontinued. It had accrued 19 patients without demonstrating antitumor activity.

At the conclusion of the study, 75 patients had been entered in the study and 73 patients were included in the analysis. Two patients, one from the Baker's antifol group and one from the standard-dose methotrexate group, never received protocol therapy (one refused participation and the other was declared ineligible prior to treatment initiation). Twenty-six patients received Baker's antifol, 28 received standard-dose methotrexate, and 19 received razoxane.

Patient characteristics are listed in table 1.

Most of the patients had a poor performance status (ECOG of 2 or 3). Only one patient from each of the completed study arms was previously untreated, two due to heart disease. Patient characteristics included: 90% with serum bilirubin ≤ 1.5 mg/100 ml and 15% with linitis plastica histology. The modal patient treated with razoxane or Baker's antifol had poorly differentiated tumors, and the modal patient treated with methotrexate had moderately differentiated tumors. There was a 3:1 male predominance; one-half of the patients had lost ≥ 5% of their body weight within the previous 6 months, and one-half had demonstrable metastasis to the liver. The median time from initial diagnosis to protocol treatment was 43 weeks with Baker's antifol, 37 weeks with methotrexate, and 28 weeks with razoxane, a significant difference ($P = 0.01$). The median ages also nearly differ statistically ($P = 0.055$); the median age of the patient who received Baker's antifol was 63 years as opposed to 55 years for the patients who received razoxane. The distribution of the patients' on-study performance status significantly differed ($P = 0.01$) among the participating institutions. One institution contributed 73% of the patients with ECOG performance status of 0 or 1.

Toxicity

Thirty-two patients experienced severe or worse treatment-related toxic effects. Of these patients, ten

TABLE 1.—Selected patient characteristics (GI-8477)

Characteristic	Treatment			
	Baker's antifol	Standard-dose methotrexate	Razoxane	Total
No. of patients	26	28	19	73
ECOG performance score				
0	3	1	1	5
1	9	10	6	25
2	8	11	7	26
3	6	6	5	17
Prior therapy				
No study agents	22	23	17	62
Razoxane	4	5	0	9
Antifolates	0	0	2	2
Bilirubin (mg/100 ml)				
≤ 1.5	26	25	15	66
> 1.5	0	3	4	7
Primary type				
Linitis plastica	6	4	1	11
Other	20	24	18	62
Differentiation				
Good	4	3	0	7
Moderate	8	15	5	28
Poor	11	10	13	34
Unknown	3	0	1	4
Sex				
Male	18	22	15	55
Female	8	6	4	18
Weight loss				
< 5% of body weight	11	8	9	28
≥ 5% of body weight	13	17	10	40
Unknown	2	3	0	5
Lung involvement				
Yes	5	6	6	17
No	19	21	10	50
Unknown	2	1	3	6
Liver involvement				
Yes	12	17	9	38
No	10	6	8	24
Unknown	4	5	2	11
Median age in yrs	63	63	55	61
Median time in wks from initial diagnosis to treatment	43	37	28	38

were receiving Baker's antifol, ten were receiving standard-dose methotrexate, and 11 were receiving razoxane. One toxic death was observed in a patient receiving standard-dose methotrexate. There were no apparent imbalances in the overall number of patients experiencing severe toxic effects in these three treatments. As expected, Baker's antifol produced some instances of severe stomatitis and body rash, and standard-dose methotrexate produced anorexia, stomatitis, and one episode of moderate renal failure (serum creatinine, 2.7 mg/100 ml). These were known potential complications of the antifolate regimens. All 11 patients experiencing severe toxicity on the razoxane arm had decreased wbc or platelet counts. Only four patients with severe myelosuppression were seen in both the Baker's antifol and standard-dose methotrexate arms. Razoxane was significantly ($P < 0.01$) more myelosuppressive by these criteria at the study's dosages.

Failure to measure creatinine clearance before randomization had no apparent impact on toxicity. Nevertheless, determination of creatinine clearance may be a useful safeguard before deciding on therapy with methotrexate.

Tumor Response To Therapy

Two partial responses and one complete response were observed in patients treated with standard-dose methotrexate. Four partial responses were observed in patients treated with Baker's antifol. Responses included complete remission of pulmonary metastases (methotrexate) and partial remission of abdominal masses (both methotrexate and Baker's antifol), liver metastases (Baker's antifol), and subcutaneous nodules (Baker's antifol).

If patients with early deaths (within 21 days) without tumor progression are removed from the denominator, the response rates are 17% (four responses among 23 patients), 12% (three responses among 25 patients), and 0% (no response among 17 patients) for Baker's antifol, methotrexate, and razoxane, respectively. If all patients are considered, response rates are 15% (four responses among 26 patients), 11% (three responses among 28 patients), and 0% (no response among 15 patients) for the same three drugs, respectively.

Response rates by ECOG status for Baker's antifol and methotrexate are 25% (one response among four patients), 21% (four responses among 19 patients), 5% (one response among 19 patients), and 8% (one response among 12 patients) for ECOG performance status 0, 1, 2, and 3, respectively. Only one response was reported from institutions that predominantly contributed patients with poor performance status.

Only 18% of the patients had responded to prior therapy. Only two responders, one on each arm, had responded to prior therapy.

Sixty-eight deaths have been observed among the 73 patients reported herein. The estimated median survival times are: 18, 9, and 8 weeks for patients treated with Baker's antifol, standard-dose methotrexate, and razoxane, respectively. The product limit estimates of the survival curves are shown in figure 1. A comparison of the three survival distributions, using both the log-rank and Gehan-Wilcoxon tests, found a difference among the three treatments ($P < 0.05$). Poor performance status (2 or 3) and substantial weight loss ($\geq 5\%$) in the previous 6 months are both significantly associated with poor prognosis ($P < 0.01$). Neither age nor time interval from diagnosis to protocol treatment influenced prognosis or affected treatment comparisons. Pair-wise comparisons of the treatment arms again demonstrate the superiority of Baker's antifol to the other treatments after adjustment for the performance status and weight loss ($P < 0.05$). Removal of patients with high

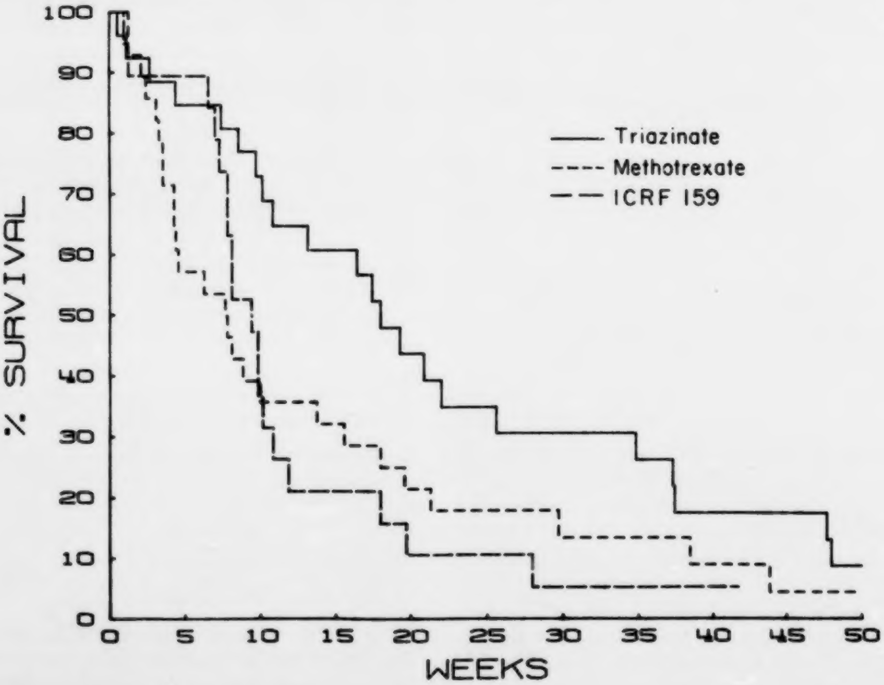

FIGURE 1.—Product limit estimates of survival curves for patients treated with Baker's antifol (triazinate), methotrexate, or razoxane (ICRF 159).

bilirubin levels (> 1.5 mg/100 ml), who were excluded by design from the Baker's antifol arm, minimally affected the significance of pair-wise tests. One-tailed tests maintained their significance. Performance status by institution did not affect the observed differences in survival by treatment.

Further analyses examined the type, duration, and results of prior chemotherapy in relation to response and survival. There were no imbalances with respect to the methotrexate and Baker's antifol groups.

DISCUSSION

The study found that Baker's antifol produces objective tumor responses and has an apparent beneficial impact on overall survival compared to standard-dose methotrexate or razoxane for patients with advanced measurable gastric carcinoma refractory to treatment with 5-FU, doxorubicin, and long-acting alkylating agents. Response rates for Baker's antifol are comparable to those previously observed with doxorubicin in patients failing prior therapy (8,11). These observations provide the rationale for advancing Baker's antifol into phase III combination therapy trials. Additional investigation on this and other antifolates including methotrexate appears warranted both to confirm these observations and to test other dose schedules.

The observed 18-week median survival following treatment with Baker's antifol appears relatively superior to that with standard-dose methotrexate or razoxane and comparable to that with doxorubicin in similar second-line treatment studies (5,8). The observed impact of Baker's antifol on survival was supported by survival comparisons, with adjustment for potentially important prognostic variables.

The apparent new findings of this study must be viewed cautiously. The survival advantage by treatment appeared to overcome prognostic considerations; however, the initial "randomization" was conditional. In theory, unrecognized factors may be responsible for a false-positive survival analysis. The finding that a second antifolate also produces objective responses would appear to support the potential efficacy of both antifolates. The failure to find an overall survival advantage for patients treated with methotrexate led the GITSG to select Baker's antifol for phase III study.

With this study, the GITSG has a positive report suggesting a role for chemotherapy in every stage of gastric cancer (8,9,12–14). Patients with a response to chemotherapy were found to have improved survival twice that of all patients with the same profile of prognostic characteristics (12). The 5-FU-semustine regimen, which was found to have no impact on survival with advanced disease, appears to improve survival in early stages of disease (13,14). These findings across all stages of gastric cancer strongly warrant continued systematic testing of new combinations and new drugs for patients with advanced gastric cancer.

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Epileptic Seizures Associated With Cisplatin Administration[1]

Graham M. Mead,* Andrew M. Arnold, John A. Green, Fergus R. Macbeth, Christopher J. Williams, and J. Michael Whitehouse[2]

Eighty-one patients with germ cell tumors or ovarian carcinoma were treated over a 4-year period with cisplatin-containing combination chemotherapy. Eight patients (9.8%) experienced epileptic seizures during or within 3 months of this chemotherapy. While a definite causal relationship between the seizures and cisplatin could not be established, we have rarely seen such episodes in relationship to other cytotoxic drugs. A number of possible contributing factors were apparent, including hyponatremia and pretreatment impairment of renal function. [Cancer Treat Rep 66:1719–1722, 1982]

Cisplatin is a widely used chemotherapeutic agent that has shown particular activity in nonseminomatous germ cell tumors and ovarian carcinoma (1,2). However, toxic effects, particularly nausea and vomiting and progressive impairment of renal function, are common (3). In addition, ototoxicity [manifest by high-tone hearing loss (4)], myelosuppression (3), and renal electrolyte wasting (particularly of Mg^{++} and Ca^{++}) are well-described (5,6). Neurologic toxicity has predominantly comprised sensory and motor peripheral neuropathy (7–12), although there are reports of seizures associated with use of the drug (12–15).

This report summarizes our experience with seizures related to cisplatin-containing combination chemotherapy and attempts to identify contributory factors in their etiology.

MATERIALS AND METHODS

Hospital charts were reviewed on all patients receiving cisplatin between August 1977 and August 1981. This drug was used exclusively to treat metastatic carcinoma of the ovary (55 patients) and germ cell tumors of the testis and ovary (26 patients). A total of 298 treatment courses were given (one to eight per patient), all in combination with other drugs. All patients were in the hospital for 24–48 hours to receive their treatment.

Chemotherapy for ovarian carcinoma (PACe) comprised cisplatin (80 mg/m^2), doxorubicin (40 mg/m^2), and cyclophosphamide (1 g/m^2), all given by iv push on Day 1 of a 28-day cycle. Cisplatin administration was preceded by an infusion of 5% dextrose in $^1/_5$ normal saline given iv at a rate of 500 ml every 2 hours for at least 2 hours and until the urine output exceeded 150 ml/hour. Cisplatin was reconstituted in water for injection. Iv fluids were continued for at least 6 hours, alternating 500 ml of normal saline with 500 ml of 5% dextrose in $^1/_5$ normal saline with potassium supplements. Iv fluids were then continued every 3 hours until emesis stopped and the patients were able to drink adequately. Doses of all drugs were modified according to peripheral blood cell counts prior to therapy. Cisplatin doses were modified according to renal function; the drug was withheld if the creatinine clearance was $<$ 35 ml/minute, a 50% dose was given for a clearance of 35–50 ml/minute, and a 100% dose was given for a clearance $>$ 50 ml/minute. These guidelines were sometimes contravened if cisplatin administration was felt to be clinically indicated.

Patients with germ cell tumors were treated with either PVB chemotherapy as described by Einhorn and Williams (16) or a modification of this therapy. PVB chemotherapy comprised cisplatin (20 mg/m^2) iv daily for 5 days, vinblastine (0.15–0.2 mg/kg) iv on Days 1 and 2, and bleomycin (15 mg/m^2) iv on Days 2, 9, and 16 of a 21-day cycle. Early in our experience with this therapy, cisplatin was given as a 100-mg/m^2 iv bolus on Day 1. A number of patients, treated as part of a national study, received etoposide at a dose of 100 mg/m^2 iv daily for 5 days in conjuction with PVB. When this regimen (BEViP) was used, the vinblastine dose was reduced to 0.15 mg/kg on Days 1 and 2. Hydration prior to cisplatin administration was as for the PACe regimen.

Patients receiving cisplatin over a 5-day period were given an iv infusion of normal saline (500 ml every 4 hours with potassium supplements) throughout the treatment period. Dose modifications for nephrotoxicity were as described for PACe chemotherapy.

Hospital charts were carefully reviewed for evidence of neurotoxicity possibly related to cisplatin administration. Neurologic events occurring within a 6-month period of drug administration were recorded and analyzed. The subsequent course of the patient was assessed to exclude, as far as possible, metastatic disease as an explanation for these events.

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[1] Received Feb 11, 1982; revised May 24, 1982; accepted June 7, 1982.

[2] CRC Medical Oncology Unit, Southampton General Hospital, England.

**Reprint requests to:* CRC Medical Oncology Unit, Southampton General Hospital, Southampton, SO9 4XY, England.

RESULTS

Neurologic Events

Eight patients (9.8%) had epileptic seizures possibly related to cisplatin therapy. In addition, two of these patients (Nos. 3 and 8) reported numbness and paresthesia in their extremities after completing chemotherapy, which was consistent with peripheral sensory neuropathy.

A further six patients (7.4%), comprising five who had ovarian carcinoma treated with PACe and one who had germ cell tumor of the testis treated with PVB, described sensory symptoms consistent with peripheral neuropathy. These symptoms occurred after therapy was completed, were not associated with motor weakness, and disappeared spontaneously within 6 months. Finally, two patients (one with ovarian carcinoma receiving PACe and one with germ cell tumor of the testis receiving PVB) had Lhermitte's syndrome following completion of therapy; the symptoms lasted approximately 3 months.

Seizures

Details of the patients who had seizures are recorded in table 1. There were five female patients (ovarian carcinoma, four; germ cell tumor of the ovary, one) and three male patients (all with germ cell tumors of the testis). Their ages ranged from 23 to 66 years.

The temporal relationship of seizures to therapy was widely variable. One patient (No. 1) developed involuntary movement of his left hand and arm during the first drug infusion. These episodes recurred on several occasions within 7 days of the first and third drug cycles. Other patients developed seizures within 8 hours, 2 days, 7 days, 2 months, and 3 months of cisplatin administration. Although seizures occurring several weeks after drug therapy cannot necessarily be attributed to cisplatin, we have been impressed that such problems occur rarely in a much larger series of patients receiving other chemotherapy regimens.

Seizures were not necessarily considered a contraindication to further cisplatin therapy, particularly if easily identifiable precipitating factors were apparent. Thus, three patients (Nos. 1–3) received additional drug despite a history suggestive of seizures early in their therapy. Patients 1 and 2 developed probable evidence of seizure activity related to two of four and three of four PVB courses, repsectively. Patient 2 was ultimately admitted to the hospital in status epilepticus.

Seizures were clearly focal in four patients (Nos. 1, 2, 5, and 7) and were associated with transitory postictal evidence of mild hemiparesis in three (Nos. 2, 5, and 7). A single patient (No. 2) has continued to have an occasional grand mal seizure and has required continued treatment with phenytoin. There were otherwise no permanent neurologic sequelae.

Investigation of Seizures

All patients who had seizures were immediately tested for abnormalities of electrolytes (including serum Ca^{++}), renal function, and blood glucose. Serum magnesium concentrations were not routinely evaluated.

No neurologic investigations were carried out in five patients, since seizures occurred only once and precipitating factors were recognizable. Two patients (Nos. 2 and 7) had head CT scans and one patient (No. 4) had an isotope brain scan with normal results. These same patients also had an EEG examination; for two (Nos. 2 and 7), an epileptic focus was found (table 1).

Precipitating Factors

In seven patients, factors that may have contributed to an epileptic tendency were apparent. These included an association with micturition and recent phenothiazine administration (patient 3; probable diagnosis of micturition syncope), probable hypoxia in a patient (No. 4) disabled by bleomycin-induced lung fibrosis, and moderately severe hyponatremia (patients 6 and 8). This was related in one case to a recent abdominal paracentesis and in both cases to severe emesis after cisplatin administration. Mild impairment of renal function was apparent before therapy in three patients (Nos. 5, 7, and 8): two developed seizures early (6 hours and 2 days after treatment) and one developed seizures after 3 months.

Another possible contributing factor was moderate thrombocytopenia at the time of a seizure (patient 5). Finally, patient 2 was receiving anticoagulants for pulmonary emboli, and at the time of admission she had evidence of a bleeding diathesis (BCR, 6.1; anticoagulant range, 2–4) and hypertension. There was no evidence of peripheral bleeding.

No clear evidence of a cumulative dose effect was apparent.

Clinical Outcome

All four patients with germ cell tumors are alive and free of tumor at 4+, 20+, 28+, and 31+ months after completion of induction therapy. Patient 6 with ovarian carcinoma died of progressive abdominal disease within 2 months of her final cisplatin therapy (no autopsy was done), and the other three patients are alive with disease at 4+, 7+, and 14+ months.

DISCUSSION

Cisplatin has proved to be a highly successful cytotoxic drug, particularly in the therapy of germ cell tumors and ovarian carcinoma (1,2). Unfortunately, a

TABLE 1.—Characteristics of 8 patients who had seizures during or after cisplatin-containing therapy

Patient No.	Age and sex	Diagnosis*	Therapy	Seizure description	Temporal relationship to therapy	Possible precipitating factors	Neurologic investigations	Outcome†
1	23 M	Stage III NSGCT	BEViP × 4	Multiple focal seizures of left hand and arm; single grand mal episode	During and up to 7 days after chemotherapy courses 1 and 3	—	—	No seizures since completed chemotherapy; $A^0$20
2	34 F	Stage III germ cell tumor of ovary	PVB × 4	1. Unexplained blackouts × 2 while recumbent 2. Focal seizures of left arm + grand mal seizures; mild left hemiparesis	1. 7 days after courses 1 and 3 2. 7 days after course 4	At time of focal seizures: 1. Receiving anticoagulant Coumadin for pulmonary emboli On admission: 2. Blood pressure 210/130 3. BUN 96 mg/100 ml	Head computerized tomographic (CT) scan normal; EEG focus right posterior temporal region	Complete recovery though occasional grand mal seizure while receiving phenytoin; $A^0$31
3	36 M	Stage III NSGCT	PVB × 4	Single grand mal seizure	7 hrs after PVB, course 3	1. Chlorpromazine im 2 hrs before seizure 2. Seizure occurred after standing to micturate 3. Postural hypotension observed	—	No further seizures; symptoms of peripheral neuropathy; $A^0$4
4	38 M	Stage II NSGCT	PVB × 5	Grand mal seizures × 2	3 and 4 mos after PVB completion	Bleomycin-induced lung fibrosis with hypoxia on exertion	Isotope brain scan normal; EEG normal	No further seizures; $A^0$28
5	55 F	Stage III ovarian carcinoma	PACe × 3	Grand mal and focal seizures involving left side; mild left hemiparesis	2 mos after PACe, course 3	On admission: 1. Platelet count 60×10^3/mm^3 2. BUN 60 mg/100 ml	—	Complete recovery, no further seizures; A^+14
6	61 F	Stage III ovarian carcinoma	PACe × 2	Grand mal seizures	2 days after PACe course 2	1. Abdominal paracentesis prior to chemotherapy (electrolytes normal at this time) 2. Serum Na^+ 109 mg/100 ml at time of seizures	—	Progressive disease; D^+2
7	64 F	Stage III ovarian carcinoma	PACe × 4	Grand mal and focal right-sided seizures; mild right hemiparesis + dysphasia	6 hrs after PACe, course 4	1. BUN 84 mg/100 ml and creatinine 1.2 mg/100 ml prior to chemotherapy due to right uteric obstruction	Head CT scan, cerebral atrophy, EEG-focus left posttemporal region	Complete recovery, no further seizures; A^+4
8	66 F	Stage III ovarian carcinoma	PACe × 3	Grand mal seizure	2 days after PACe, course 3	1. BUN 50 mg/100 ml and creatinine 1.3 mg/100 ml prior to chemotherapy 2. Serum Na^+ 112 mg/100 ml at time of seizure	—	No further seizures, symptoms of peripheral neuropathy; A^+7

*NSGCT = nonseminomatous germ cell tumor.

†A^0 = alive with no clinical evidence of disease; A^+ = alive with clinical evidence of disease; and D^+ = dead with evidence of disease. Values = No. of mos.

number of toxic effects are common (1–15) and make the drug intolerable to some patients.

Toxicity to the nervous system is now well-described and is commonly manifested by sensory peripheral neuropathy, occasionally progressing to motor involvement (7–12). Figures indicating the incidence of this complication are not available; however, subclinical evidence of nerve damage with increasing dose has not been uncommon in prospective nerve conduction studies (8).

Other reports of neurologic damage have included rare patients who have developed papilledema (10,17), retrobulbar neuritis (17), low pressure hydrocephalus (10), and transient cortical blindness (15).

An association between cisplatin administration and seizures has been alluded to in a number of studies (12–15), although few detailed case reports are available. The series reported in this study is the largest in the literature, with almost 10% of the patients who received cisplatin developing clinical evidence of seizures within 3 months of therapy. It is possible that our rather broad inclusion criteria were partly responsible for this high incidence.

Close examination revealed a number of possible precipitating factors, some of which may have entirely accounted for the seizure activity. In two of our cases moderately severe treatment-related hyponatremia (known to be a cause of seizures) was present. In four cases there was evidence of impairment of renal function either at the time of cisplatin therapy or subsequently when seizures developed. Although insufficient to explain the seizures, it is possible that high peak levels or prolonged CNS exposure to this drug lowers the threshold at which these events might occur.

None of the other chemotherapeutic drugs used in these patients has been described as causing seizures. We were unable to demonstrate evidence of cerebral metastases either at the time of evaluation or at followup of these patients. Serum magnesium levels were not routinely evaluated. Although hypomagnesemia resulting from cisplatin administration has been well-described (5,6), seizures as a result of this complication have not been reported. Nevertheless, hypomagnesemia may have been a contributing factor (18,19) and should be looked for in this setting.

In most of our patients, seizures occurred only once and did not require long-term antiepileptic therapy. We have not regarded only one seizure as a contraindication to further therapy with cisplatin, although this decision must be taken on an individual patient basis in conjunction with other clinical factors.

In conclusion, we have described an association between the administration of cisplatin and the development of seizures in patients with germ cell tumors and carcinoma of the ovary. Seizures have occurred at any time from 6 hours to 3 months after therapy and although no definite causative relationship could be established it does seem likely that cisplatin lowered the seizure threshold. In some cases this may be sufficient to precipitate seizures, while in others the coexistence of other factors may be necessary. This study indicates that careful attention to electrolyte balance, particularly where vomiting is severe or protracted, is most important. Additionally, moderate impairment of renal function may have contributed to the possibility of seizures and should be regarded as a further relative contraindication to further use of the drug in this setting.

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High-Dose Allopurinol Modulation of 5-FU Toxicity: Phase I Trial of an Outpatient Dose Schedule[1,2]

Thomas N. Campbell,[3,*] Stephen B. Howell,[4] Craig Pfeifle,[4] and Barbara A. House[3]

In an attempt to decrease the activation of 5-FU by normal cells relative to cancer cells, 20 patients with metastatic cancer were given 72 courses of 5-FU and allopurinol (HPP) in a phase I trial. 5-FU was given daily by iv bolus injection for 5 consecutive days every 4 weeks; HPP, 300 mg orally every 8 hours for 6 consecutive days, was started 24 hours before the first injection of 5-FU. HPP appeared to modulate 5-FU toxicity by allowing higher doses (18–21 mg/kg daily for 5 days) to be given. Unexpectedly, neurotoxicity was the dose-limiting toxicity; it was slowly reversible and manifested primarily as encephalopathy, with some patients also having cerebellar signs. Gastrointestinal and hematologic toxic effects were mild and infrequent. Because of the high incidence of neurotoxicity and low response rate, this program does not appear to offer any advantages over conventional dose schedules of 5-FU alone. [Cancer Treat Rep 66:1723–1727, 1982]

Allopurinol (HPP) given simultaneously with 5-FU has been shown to decrease 5-FU toxicity for cells in tissue culture (1), for rodents (2), and for man (3,4). Tumor response rates are proportional to the 5-FU dose rate (5). The rationale for the concurrent use of 5-FU and HPP is that if HPP selectively protects normal tissues, then higher dose rates of 5-FU could be tolerated, and greater tumor response rates would be anticipated.

5-FU is converted intracellularly to two active metabolites, 5-fluorodeoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FdUMP is a potent inhibitor of thymidine synthetase, and FUTP is incorporated into several species of RNA, interfering with subsequent function (6,7). The first step in the formation of these active metabolites is the conversion of 5-FU to FUMP (fig 1). This can occur via either the action of orotidylate phosphoribosyltransferase (OPRTase) or the sequential action of uridine phosphorylase and uridine kinase. HPP is converted by xanthine oxidase to oxipurinol, which is phosphorylated to 1-oxipurinol-5′-monophosphate (8), a potent inhibitor of orotidylate decarboxylase (ODCase) (9). Inhibition of ODCase results in enhanced formation of orotidine, causing inhibition of OPRTase and increasing the intracellular concentration of orotic acid. Orotic acid effectively competes with 5-FU for OPRTase (10), thus decreasing the formation of FdUMP and FUTP via the OPRTase pathway. HPP can potentially increase the selectivity of 5-FU if normal tissues depend primarily on the OPRTase pathway and malignant tissues on the uridine phosphorylate-kinase pathway for activation of 5-FU. In addition, the activity of OPRTase has been shown to be higher in colonic adenocarcinoma cells that are sensitive to the cytotoxic effect of 5-FU than in surrounding normal colonic mucosal cells (11). Thus, HPP could potentially increase the selectivity of 5-FU if the HPP dose rate could be titrated to inhibit the activity of OPRTase in normal cells; then, those adenocarcinoma cells with excess OPRTase activity should still be able to convert 5-FU to FdUMP and FUTP.

Two phase I trials have been reported indicating that HPP can reduce the toxicity of 5-FU in man. Fox et al (3) observed three complete and three partial responses among 12 patients (ten with colorectal carcinoma and two with gastric carcinoma). Only six of their 20 patients had had previous chemotherapy. In contrast, Kroener et al (12) found no responses in 14 patients with colorectal carcinoma, 11 of whom had received prior 5-FU therapy. Both of these trials were conducted using a 5-day constant infusion of 5-FU, a schedule requiring hospitalization. Therefore, we conducted a phase I trial of HPP in combination with 5-FU administered as a single iv bolus for 5 consecutive days of each month. The aim of this trial was to determine whether concurrent treatment with HPP would permit escalation of the 5-FU dose commonly used in the outpatient setting.

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[1] Received Nov 30, 1981; revised June 14, 1982; accepted June 28, 1982.

[2] Supported in part by Public Health Service grant CA-23100 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Clayton Foundation for Research, California Division. Dr. Howell is a Clayton Foundation investigator.

[3] Kaiser Permanente Medical Center, San Diego, CA.

[4] Departments of Medicine and Pharmacy, and the Cancer Center, University of California, San Diego at La Jolla.

**Reprint requests to:* Thomas N. Campbell, MD, 4647 Zion Ave, San Diego, CA 92120.

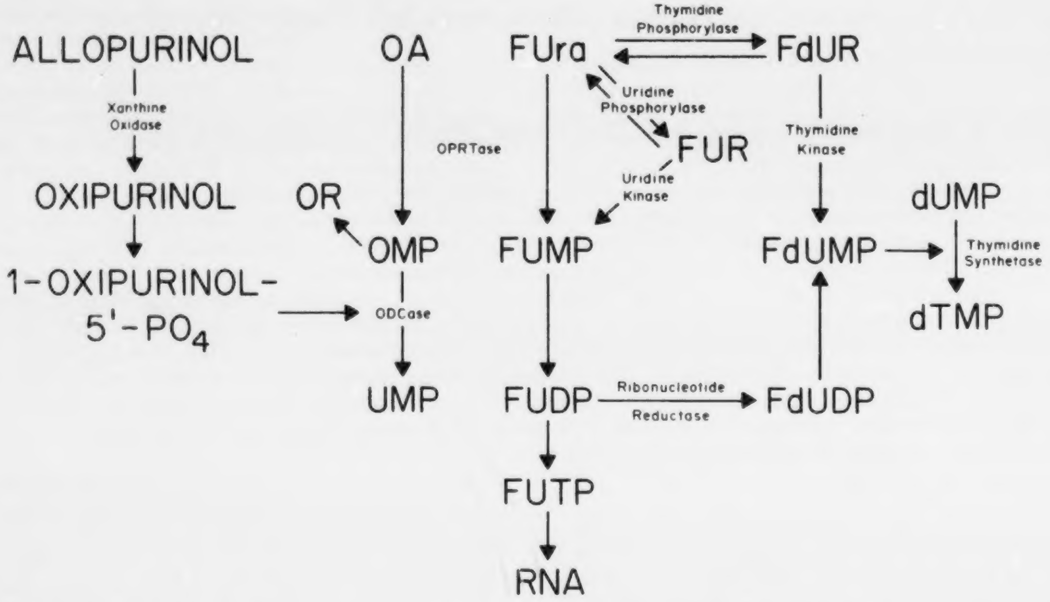

FIGURE 1.—Pathways of HPP, orotic acid, and 5-FU metabolism. OA = orotic acid; OMP = orotidine monophosphate; OR = orotidine; UMP = uridine monophosphate; FUra = 5-FU; FUR = 5-fluorouridine; FUMP = 5-fluorouridine monophosphate; FUDP = 5-fluorouridine diphosphate; FdUR = 5-fluorodeoxyuridine; FdUDP = 5-fluorouridine diphosphate; dUMP = deoxyuridine monophosphate; dTMP = thymidine monophosphate.

MATERIALS AND METHODS

Patients

Twenty adults with histopathologically proven advanced metastatic cancer, who were able to ingest oral medications, had recovered from toxic effects related to prior therapy, and had a life expectancy of at least 2 months, gave their informed consent to participate in this study. Twelve patients had colorectal adenocarcinoma; two had lung adenocarcinoma; one each had renal, adrenal, ovarian, breast, and pancreatic adenocarcinomas; and one had melanoma. Criteria for entry in the study included serum creatinine ≤ 1.5 mg/dl, leukocyte count ≥ 4000/mm^3, platelet count ≥ 150,000/mm^3, serum bilirubin ≤ 3 mg/dl, alkaline phosphatase no more than twofold elevated, and SGOT no more than threefold elevated.

Study Design and Treatment Plan

HPP, 300 mg orally every 8 hours, was started 24 hours before the first iv bolus injection of 5-FU and was continued for 6 days. The same 5-FU dose was given daily for 5 days unless significant toxicity was observed. The starting dose of 5-FU for all patients was 15–18 mg/kg/day. Treatment was repeated every 4 weeks, the condition of the patient permitting. If no or minimal body weight to a maximum of 24 mg/kg. Dose escalation was carried out both within and between patients. The HPP dose remained constant at 300 mg every 8 hours for 6 days. Measurements of disease, performance status, cbc, platelet count, serum creatinine or BUN, SGOT, bilirubin, and tumor marker were performed prior to each course of treatment. In addition, patients were examined for mucositis, cerebellar signs, and gross visual acuity prior to each treatment course and more frequently if symptoms of toxicity occurred. In reporting the hematologic toxicity, the geometric rather than arithmetic mean was used to reduce the variance. A partial response was defined as a ≥ 50% decrease in the product of perpendicular diameters of measurable lesions. Stable disease was recorded when measurable lesions decreased by < 50% or increased by < 25% in the absence of new lesions. Duration of response was considered from the time response was achieved.

RESULTS

Toxicity

Table 1 lists the hematologic values obtained 2 weeks after a course of 5-FU and HPP, as a function of the 5-

TABLE 1.—Hematologic toxicity of 5-FU administered with HPP

Dose level (mg/kg)	No. of patients	No. of courses	Geometric mean at Day 15*		No. of courses/No. evaluable	
			Wbc count (range)	Platelet count (range)	Wbc count $< 2 \times 10^3/mm^3$	Platelet count $< 100 \times 10^3/mm^3$
9	2	4	8.0 (5.3–12)	342 (182–663)	0/4	0/4
15	10	25	7.0 (3.4–12.4)	307 (101–828)	0/23	0/22
18	17	36	4.3 (0.5–10.7)	157 (48–545)	3/36	9/32
21	5	7	3.1 (0.4–5.2)	101 (63–171)	2/7	2/6
24	1	1	1.3	76	1/1	1/1

*Cells × $10^3/mm^3$.

FU dose. Two patients could not tolerate the 15-mg/kg dose because of neurotoxicity; one was given three courses and the other was given one course at the 9-mg/kg dose. The Day-15 geometric mean counts fell progressively with increasing 5-FU dose, while there was an increase in the percent of courses in which the leukocyte count fell to $< 2000/mm^3$ or the platelet count fell to $< 100{,}000/mm^3$. However, myelosuppression was not dose-limiting up to 21 mg/kg, the highest dose for which there were sufficient numbers of courses to make any statement.

Table 2 shows the nonhematologic toxic effects observed during and after each 5-FU and HPP course, as a function of 5-FU dose. When mucositis, nausea, vomiting, and diarrhea occurred they were usually mild and resolved quickly. Nausea, and less often vomiting, tended to occur during the latter days of the 5-FU injections. Mucositis and diarrhea resolved within 2 weeks after the course of 5-FU. These toxic effects were not clearly related to dose, and they were not dose-limiting.

Neurotoxicity occurred with greater frequency than either hematologic or gastrointestinal toxicity and was dose-limiting. Neurotoxic symptoms were usually mild to moderate and consisted of intermittent blurring of vision without a change in visual acuity, decreased sense of balance or "dizziness" that was often associated with an abnormal tandem gait, a positive Romberg test, nystagmus, and abnormal finger-to-nose or heel-to-shin coordination tests. Postural light-headedness, tinnitus, depression, weakness, fatigue, staggering gait (ataxia), shakiness (tremulousness), and slurring of speech were less common symptoms. The onset of neurotoxicity was frequently delayed, and recovery required weeks rather than days. As severity increased, the interval between 5-FU treatments had to be increased from 4 to 6 weeks in most cases. Because of the importance of the dose-limiting neurotoxicity in this study, case histories of two of the most severely affected patients are described in detail.

Case Reports

Case 1.—A 65-year-old man was admitted to the hospital because of increasing urinary and fecal incontinence, inability to stand or walk, and delirium of 1 week's duration. Pulmonary and liver metastases from colon adenocarcinoma had been present for $1^1/_2$ years, but he had received no therapy prior to entering this trial. He had received 5-FU (24 mg/kg, iv bolus) for 5 days, with HPP starting 21 days previously. This was his 12th monthly course of 5-FU and HPP but his first at the 24-mg/kg dose level. He had tolerated treatment at the 21-mg/kg dose level starting 7 weeks prior to admission, without any signs of neurotoxicity. Physical examination at admission showed his vital signs to be normal except for a temperature of 39°C. The liver was 14 cm in span. He was disoriented to time, place, and person but was conscious and responsive to noises and moving objects. His affect was bland; judgment, abstraction, and short-term memory were nil. Coordination and sensory and motor testing were limited by poor patient cooperation. No nystagmus was elicited. Deep tendon reflexes were intact and plantar reflexes equivocal. His hemoglobin was 9 g/100 ml, leukocyte count was 1300/

TABLE 2.—Nonhematologic toxicity of 5-FU administered with HPP

Dose level (mg/kg)	No. of patients	No. of courses	Toxic effect*			
			Mucositis	Diarrhea	Nausea/vomiting	Neurotoxicity
9	2	4	0/4	0/4	0/4	2/4
15	10	24	3/24	3/24	4/24	6/23
18	17	36	10/36	5/36	4/36	17/36
21	5	7	1/7	0/7	1/7	5/7
24	1	1	0/1	1/1	0/1	1/1

*Values = No. of courses with toxic effect/No. evaluable.

mm^3 with 1% neutrophils, and platelet count was 172,000/mm^3. Serum electrolytes, creatinine, sugar, calcium, bilirubin, bromide, vitamin B_{12}, and thyroxine were within normal limits. Lumbar puncture showed normal pressure and clear, colorless cerebrospinal fluid (CSF); no cells were seen. The CSF glucose was 72 mg/dl and the protein was 20 mg/dl. A computerized axial tomographic brain scan showed no masses. An EEG showed a grade I dysrhythmia. In 1 week the neutropenic fever responded to antibiotics and the leukocyte count returned to normal. The patient's mental status improved; he became calm, oriented, and able to remember recent events. He ambulated with minimal assistance and was discharged from the hospital. He was readmitted 2 weeks later because of intermittent delirium, agitation, disorientation, and continued urinary and fecal incontinence. These symptoms persisted without further improvement until death 3 months later. His neurologic deterioration was attributed to his treatment with 5-FU and HPP.

Case 2.—A 62-year-old man was admitted to the hospital because of increasing mental confusion and urinary incontinence for 1 week and chills and fever for 2 days. Liver metastases from colon adenocarcinoma had been diagnosed 2 years previously. Intermittent chemotherapy with 5-FU had been started 11 months previously but was stopped 3 months before the patient entered this trial. His second course of 5-day 5-FU and HPP chemotherapy, administered at a dose of 18 mg/kg/day, had been started 3 weeks before this hospital admission. During examination, his temperature was 39.4°C, pulse was 100/minute, and blood pressure was 100/60. The liver span was 10 cm. He was conscious but moderately confused and agitated. His affect was flat. He could answer yes-no questions appropriately. Within the limits of his cooperation the motor, sensory, cerebellar, and cranial nerve examinations were normal and there were no localizing neurologic signs. The hemoglobin was 11 g/100 ml, leukocyte count was 1500/mm^3 with 2% neutrophils, and platelet count was 76,000/mm^3. Serum electrolytes, glucose, BUN, amylase, and calcium were within normal limits. A lumbar puncture showed normal pressure and clear, colorless CSF with glucose of 77 mg/dl, protein of 42 mg/dl, four rbcs/mm^3, no leukocytes, and negative cytology and bacterial culture. The patient was given iv carbenicillin and gentamicin. Within 2 days he was afebrile. His neutrophil and platelet counts rose to normal. His mental status improved over the 5 days he spent in the hospital. He became oriented, calm, ambulated without assistance, and became capable of self-care. Over the next 3 weeks all symptoms and signs of neurotoxicity remitted.

Response

All 20 patients entered in this trial had measurable disease. One patient with colon carcinoma (case 1) had a partial response of 11 months' duration; he had not received previous chemotherapy. Three other patients with colorectal adenocarcinoma had stable disease lasting 4–7 months. Eight of the patients had received 5-FU chemotherapy prior to entering this 5-FU-HPP study.

DISCUSSION

The maximum tolerated dose of 5-FU is generally accepted to be 12–15 mg/kg given by iv bolus injection for 5 consecutive days, then one-half dose every other day until toxic effects develop (5). Doses as high as 19.2 mg/kg for 5 days have been given to selected individuals (13). In man, as in mice, the coadministration of HPP appears to partially protect the bone marrow and gastrointestinal tract against 5-FU-induced toxicity (3,4). This protective effect allows larger doses of 5-FU to be given when a constant-infusion schedule is used (12). Whether HPP can afford significant protection against single or multiple iv bolus doses of 5-FU is less clear. It was not possible to fully evaluate the effect of HPP on marrow and gut toxicity in this study because of the severity of the neurotoxicity. In another study from this institution (14), we found that when 5-FU was administered concurrently with HPP, or following only 24 hours of HPP pretreatment, no significant protection against myelosuppression was observed. However, it has recently been reported that a 4-day period of HPP pretreatment did afford some protection, and nadir leukocyte counts were significantly higher when the two drugs were given together (15). Thus, with longer periods of pretreatment it may be possible to escalate the dose of 5-FU given on a bolus regimen. Currently available evidence suggests that it is unlikely that HPP will permit more than a doubling of the 5-FU dose.

Neurotoxicity has been observed in all the reported trials of 5-FU in combination with HPP (3,4,12,15) and was dose-limiting in this study. In studies from this institution, the incidence of neurotoxicity was greater when 5-FU was used on an intermittent bolus injection schedule than when it was given as a 5-day constant infusion (4,12,14). This suggests that the very high plasma 5-FU concentrations that are achieved briefly with bolus injections contribute importantly to the development of neurotoxicity. The symptoms and signs of neurotoxicity were similar to those that have been reported previously with high dose rates of 5-FU without HPP (16–18) and were predominantly encephalopathic and cerebellar. The incidence, however, was much higher in this series (31 of 71 courses, 44%), and it increased with dose; however, it should be emphasized that, because of the delayed onset and prolonged duration of the neurotoxic symptoms, it was not always possible to discriminate between the effect of cumulative drug dose and the effect of dose escalation. This is in contrast to the two previously reported series (3,4) using continuous 24-hour iv infusions of 5-FU with HPP (300 mg every 8 hours orally). The incidence of neurotoxicity in the previous studies was lower: one of 20 (4) and three of 23 (3) patients. The clinical manifestations of neurotoxicity associated with the constant-infusion 5-FU dose schedule were also different, with a predominance of seizures and the absence of cerebellar signs or symptoms.

Neurotoxicity has been observed in other programs in which 5-FU has been combined with a modulating agent. Thymidine by itself causes neurotoxicity only when given at doses ≥ 75 g/m^2/day for 5 days (19). By contrast, when 15 g of thymidine was infused over 20 minutes followed 1 hour later by 5-FU at doses of 7.5–10 mg/kg by iv bolus, ten of 13 patients who received more than two courses developed symptoms and/or signs of neurotoxicity (20). Two of 16 patients required hospitalization for "cerebellar dysfunction." Central nervous system symptoms have also been observed with the combination of 5-FU and PALA; mild to moderate lethargy was common (21). The addition of thymidine to 5-FU and PALA produced a 13% incidence of drowsiness, and a 10% incidence of weakness that were not dose-related (22). There is little information on the biochemical basis for the neurotoxicity of 5-FU (23,24). However, the fact that these modulating agents affect the de novo pathway of pyrimidine synthesis and the observation that other drugs inhibiting the same pathway such as 6-azauracil produce a similar pattern of neurotoxicity (25) suggest a central role for pyrimidine metabolism in the neurotoxicity of 5-FU.

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Phase II Study of Vindesine in Patients With Advanced Breast Cancer[1,2]

Brian K. Walker,[3,*] Peter C. Raich,[3] Joseph Fontana,[3] V. P. Subramanian,[3,4] John S. Rogers, II,[3] James A. Knost,[3,5] and Beth Denning[3]

Vindesine, a new vinca alkaloid, was administered to 20 patients with advanced refractory breast cancer in a phase II trial. The drug was given at a dose of 3 mg/m^2 by iv bolus each week for 6 consecutive weeks, and responding patients were maintained on a dose of 4 mg/m^2 every 2 weeks. Nineteen patients were evaluable for disease response; partial remissions were obtained in five patients, for a response rate of 26%. Leukopenia was the major dose-limiting toxic reaction, but most patients were able to tolerate this schedule without difficulty. Neurotoxicity was mild and did not require dose reduction. [Cancer Treat Rep 66:1729-1732, 1982]

Vindesine is a new semisynthetic vinca alkaloid with structural similarity to vinblastine. Preclinical studies in animal tumor systems have demonstrated a spectrum of antitumor activity wider than that of vinblastine and very similar to that of vincristine. However, in the preclinical investigations, vindesine has produced fewer neurotoxic reactions than vincristine and less myelosuppression than vinblastine (1).

Initial phase I-II clinical studies have examined the efficacy and toxicity of vindesine administered by iv bolus at 1-2 week intervals. The major dose-limiting toxic effect in most phase I trials has been leukopenia. Mild to moderate neurotoxicity was frequently noted, but seldom required dose reduction. Patients receiving the drug on a weekly schedule have tolerated a dose of 2-4 mg/m^2 without excessive side effects (2).

The results of early phase II trials indicate that vindesine has significant antitumor activity in breast cancer. Four published phase II studies have demonstrated a response rate of 18%-29% when the drug is given on a weekly iv schedule (3-6). However, not all investigators have observed this degree of activity, with two trials producing a response rate of only 0%-4% (7,8).

This report presents the results of a phase II trial of vindesine in advanced breast cancer using a dose and schedule similar to prior studies. We are able to confirm the activity of vindesine in refractory breast cancer.

METHODS

Twenty patients with histologically proven breast cancer and recurrent disease were entered in this study. All patients had failed prior combination chemotherapy and those treated with hormonal therapy had demonstrated progressive disease after withdrawal of the hormonal agent. At least one site of measurable/evaluable disease was required. Other criteria for entry in this protocol included age < 70 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, normal hepatic and renal function, a leukocyte count $> 3000/\text{mm}^3$, and a platelet count $> 50{,}000/\text{mm}^3$. All patients were required to sign an informed consent statement and be geographically accessible to followup.

Vindesine was supplied by Eli Lilly and Co. The treatment schedule consisted of vindesine (3 mg/m^2), given by iv bolus for 6 consecutive weeks as tolerated by blood cell counts and toxic reactions. Patients with evidence of disease response or stable disease at the completion of the initial 6-week induction phase were then maintained on a dose of 4 mg/m^2 every 2 weeks until evidence of disease progression. The following sliding scale was used for dose adjustment based on a cbc performed on the day of treatment: for wbc count $> 4000/\text{mm}^3$ and platelet count $> 100{,}000/\text{mm}^3$, 100% dose; for wbc count of 3000-4000/mm^3 or platelet count of 50,000-100,000/mm^3, 50% dose; for wbc count $< 3000/\text{mm}^3$ or platelet count $< 50{,}000/\text{mm}^3$, therapy was delayed until the blood cell counts improved.

All patients were seen on a weekly basis and evaluated for both drug toxicity and disease response. Preliminary studies prior to treatment included a complete history and physical examination, cbc with differential, platelet count, urinalysis, serum chemistry profile, chest X-ray and X-rays of indicator lesions, bone scan, liver scan, and biopsy of recurrent disease as appropriate.

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[1] Received Apr 5, 1982; accepted June 16, 1982.

[2] Presented in part at the 12th International Congress of Chemotherapy, Florence, Italy, July 19-24, 1981.

[3] Department of Medicine, West Virginia University School of Medicine, Morgantown.

[4] *Present address:* Department of Medicine, Olin E. Teague Veterans Administration Hospital, Temple, TX.

[5] *Present address:* Biological Response Modifiers Program, National Cancer Institute, Frederick Cancer Research Center, MD.

[*] *Reprint requests to:* Brian K. Walker, MD, Department of Medicine, West Virginia University School of Medicine, Morgantown, WV 26506.

Followup evaluation during therapy included a weekly cbc and platelet count as well as assessment of disease response and drug toxicity. Studies performed at 4-week intervals included a serum chemistry profile, chest X-ray and X-rays of indicator lesions, and liver scan when indicated. Repeat bone scans were performed at 12-week intervals as appropriate.

The criteria for evaluating disease response were defined as follows: *complete response (CR)*— the complete regression of all measurable disease, *partial response (PR)*—a decrease of at least 50% of the tumor area in all involved sites for a minimum of 4 weeks, and *progressive disease*—a 25% increase in the area of any existing lesion or the appearance of a new lesion. Stable disease status was assigned to patients not meeting these criteria. Response duration was calculated from the beginning of chemotherapy until evidence of progressive disease.

RESULTS

Twenty patients were entered in this study and all had received prior chemotherapy. Nine patients had demonstrated progressive disease while receiving prior vincristine therapy. No patient received prior therapy with vinblastine. Ten of the 20 patients had received previous hormonal therapy and demonstrated subsequent progressive disease. Details of the patient characteristics are listed in table 1.

Only one patient completed < 4 weeks of therapy due to an early death (1 week) and therefore 19 of the 20 patients were evaluable for disease response and drug toxicity. The initial 6-week cycle of vindesine therapy was well-tolerated, with 18 of the 19 evaluable patients completing this phase of the treatment. One patient received only four weekly treatments due to early progressive disease. After the initial 6-week cycle, all patients with disease response or stable disease were treated every 2 weeks with a dose escalation to 4 mg/m^2. The median number of treatments administered was ten (range, four to 25). The number of treatments administered with a dose reduction due to toxicity was minimal (median, two; range, none to 11).

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total in study	20
Menopausal status	
Premenopausal	2
Postmenopausal (natural)	15
Postmenopausal (surgical)	3
ECOG performance status	
0	5
1	9
2	6
Disease-free interval (yrs)	
< 1	5
1–3	11
3–5	2
> 5	2
Sites of disease	
Osseous	16
Soft tissue/nodes	14
Lung	7
Liver	2
Previous chemotherapy	20
Previous hormonal therapy	10
Previous vinca alkaloids	9
Age in yrs	
Median: 61	
Range: 49–69	

There were no CRs, but five patients achieved a PR, for a total response rate of 26%. Sites of disease response included soft tissue (two), osseous (two), and lung (one). The duration of disease response ranged from 14 to 56 weeks. Two of the five PRs were in patients who had failed prior vincristine therapy. Details of the responding patients are listed in table 2.

The major toxic reaction encountered in this study was leukopenia. Fifteen of the 19 evaluable patients had at least one episode of leukopenia (wbc count < 4000/mm^3). However, only one patient had severe leukopenia with a wbc count nadir of 1600/mm^3, and no patient developed infection or sepsis related to the myelosuppression. Mild to moderate anemia developed in 89% of the patients but did not require dose reduction. Only one patient developed a platelet count of < 100,000/mm^3, and no bleeding complications were noted. An interesting finding was that 15 patients manifested an

TABLE 2.—Responding patients

Patient No.	Response	Site of disease	Duration of response (wks)	Prior vinca alkaloids
1	PR	Lung	18	Vincristine
2	PR	Soft tissue	36	No
3	PR	Soft tissue	14	No
4	PR	Osseous	16	Vincristine
5	PR	Osseous	56	No

TABLE 3.—Vindesine toxicity

Toxic effect	No. of patients (%)
Leukocyte count (cells/mm^3)	
> 4000	4 (21)
3000–4000	4 (21)
2000–3000	10 (53)
1000–2000	1 (5)
Hemoglobin (g/dl)	
> 11	9 (47)
10–11	8 (42)
< 10	2 (11)
Platelet count (cells/mm^3)	
> 100,000	18 (95)
< 100,000	1 (5)
Increase of > 100,000	15 (79)
Neurotoxic effects	
Paresthesias	15 (79)
Weakness	10 (53)
Constipation	6 (32)
Miscellaneous	
Alopecia	4 (21)
Nausea/vomiting	4 (21)
Skin rash	1 (5)
Fever	1 (5)

increase in platelet count of at least 100,000/mm^3 above baseline during vindesine therapy.

The neurotoxic effects encountered in this study were mild and did not require dose reduction in any patient. Peripheral neuropathy (79%) and generalized weakness (53%) were common, with constipation noted by 32% of the patients. Miscellaneous side effects included alopecia (21%), nausea and vomiting (21%), and skin rash and fever occurring in one patient each. Details of vindesine toxicity are listed in table 3.

DISCUSSION

The response rate of 26% observed in this study of patients with refractory breast carcinoma compares favorably to other phase II trials of vindesine at similar dose schedules. The dose selected for this trial was based on reports from earlier studies which suggested that the drug was most effective when administered on a weekly schedule, and that doses > 3 mg/m^2 produced myelosuppression and neurotoxicity requiring delay in the treatment program (2,9). The dose selected for this trial was well-tolerated, with 18 of the 19 evaluable patients completing the initial 6-week induction phase. Furthermore, the median number of treatments administered was ten, with dose reduction due to toxicity required in only 31% of the treatments. Interruption of the planned treatment schedule due to severe myelosuppression was seldom necessary.

The five PRs observed in this study were in heavily pretreated patients. All responding patients had received both prior chemotherapy and hormonal therapy. In addition, two of the five patients had demonstrated progressive disease on previous vincristine therapy, supporting the contention that vindesine is non-cross-resistant with other vinca alkaloids (4,5).

Phase II trials of vindesine in breast carcinoma, using a weekly iv bolus schedule, have been reported from seven institutions, including our own. These studies are summarized in table 4. A total of 136 patients were evaluable for disease response, with CRs in three and PRs in 23, for a total response rate of 19.1%. The two trials that documented a poor response rate involved larger doses with subsequent toxicity and delay in the treatment schedule. The five phase II trials demonstrating an improved response rate had the common feature of a lower dose (3 mg/m^2/week) with less schedule interruption. The antitumor activity of vindesine in breast carcinoma is apparently improved with the lower dose administered on a regular weekly schedule.

The major toxic effect encountered in this phase II trial was leukopenia, with 15 of 19 patients (79%) experiencing at least one leukocyte count nadir < 4000/mm^3. However, only one patient had a leukocyte count < 2000/mm^3, and no episodes of infection or sepsis were encountered. Mild to moderate anemia was observed in ten of 19 patients (53%), but dose reductions were not required. Thrombocytopenia was noted in only one patient, and in most cases an increase in platelet count was observed. Fifteen of the 19 evaluable patients (79%) demonstrated an increase in platelet count of at least 100,000/mm^3 above baseline during the course of vin-

TABLE 4.—Phase II trials of vindesine in breast cancer (weekly iv bolus schedule)

Investigator (ref No.)	Dose (mg/m^2/wk)	No. of evaluable patients	No. of CRs	No. of PRs	Response rate
Smith et al (3)	3	21	2	4	6/21 (29%)
Cobleigh et al (4)	3	21	0	6	6/21 (29%)
Robins et al (5)	3	31	1	5	6/31 (19%)
Miller et al (6)	3	11	0	2	2/11 (18%)
Ohnuma et al (7)	2–5	8	0	0	0/8 (0%)
Currie et al (8)	3–4	25	0	1	1/25 (4%)
Walker et al (this report)	3	19	0	5	5/19 (26%)
Total		136	3	23	26/136 (19.1%)

desine therapy. This relative platelet-sparing effect for vindesine has been noted by other investigators (3,10).

Neurotoxicity was relatively mild and did not require dose reduction in any patient. Paresthesias and generalized weakness were reported by most patients, and approximately one third noted some constipation. Other miscellaneous side effects were less common and included alopecia, nausea, vomiting, skin rash, and fever.

In conclusion, vindesine is an active agent in advanced breast cancer, with a response rate of 26% observed in this phase II trial. The dose-limiting toxic reaction was mild to moderate leukopenia. A review of the published phase II results for vindesine in breast cancer suggests that the agent is effective when administered on a regular weekly schedule at a relatively low dose.

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Pharmacokinetics of Methotrexate and 7-Hydroxymethotrexate Following Infusions of High-Dose Methotrexate[1,2]

Henning Breithaupt[3,*] and Eberhard Küenzlen[4,5]

The pharmacokinetics of methotrexate (MXT) and 7-hydroxymethotrexate (7-OH-MTX) has been studied in seven patients treated for osteosarcoma with up to 27 cycles of MTX at doses of 140–350 mg/kg of body weight. The distribution volume of MTX was 0.186 ± 0.062 liter/kg. Peak plasma levels ranged from 540 to 1700 μM for MTX and from 12 to 560 μM for 7-OH-MTX. The MTX metabolite 2,4-diamino-N^{10}-methylpteroic acid was occasionally detectable in plasma and urine at a level of 10^{-7}M. Plasma disappearance of MTX was biphasic, with a terminal half-life of 2.1 ± 0.6 hours (mean ± SE). Plasma decay of 7-OH-MTX was mainly monoexponential, with a half-life of 23.8 ± 15.2 hours. The renal clearance of MTX (0.0623 ± 0.0232 liter/kg/hour) accounted for about 84% of the total clearance of MTX (0.0742 ± 0.0288 liter/kg/hour). In urine, 70%–94% of the dose was recovered as MTX and 0.4%–11% as 7-OH-MTX. The renal clearance of 7-OH-MTX was in the range of 0.0173 ± 0.0149 liter/kg/hour. The pharmacokinetics of MTX and 7-OH-MTX was found to be highly reproducible intraindividually. The kinetics of MTX and 7-OH-MTX was influenced by orally coadministered activated charcoal, presumably by inhibition of enteral reabsorption of MTX and 7-OH-MTX. **[Cancer Treat Rep 66:1733–1741, 1982]**

High-dose methotrexate (MTX) (> 1 g/m^2) with leucovorin rescue is widely used as adjuvant therapy for osteosarcoma (1,2). Drug-related deaths secondary to high-dose MTX and citrovorum factor administration have been reported to occur in 6% of patients, mainly because of severe myelosuppression or renal failure (3). The safety of high-dose MTX was increased by hydration and alkalinization (4,5). Routine monitoring of plasma MTX concentrations is recommended for early detection of patients at a high risk of toxicity, who will require increased doses and prolonged courses of leucovorin (5,6).

A specific and sensitive high-pressure liquid chromatography (HPLC) method has been developed (7) for routine monitoring and pharmacokinetic studies. This paper presents the characteristics of plasma disappearance and urinary excretion of MTX and its main metabolite 7-hydroxymethotrexate (7-OH-MTX) in seven patients treated for osteosarcoma with high-dose MTX.

MATERIALS AND METHODS

Therapeutic Regimen

Seven patients with osteosarcoma (16–48 years of age) were treated with high-dose MTX between December 1977 and July 1981. The patients received up to 27 drug cycles of high-dose MTX every 3–4 weeks. A total of 122 cycles were given.

MTX doses of 140–350 mg/kg of body weight were administered by continuous iv infusion over 6 hours followed by citrovorum factor rescue. Seven doses of citrovorum factor (15 mg each) were injected iv every 6 hours, beginning 3 hours after completion of the MTX infusion. Iv fluids (5% glucose) were administered at a rate of 3500 ml/24 hours with 70 mEq of $NaHCO_3$/liter, starting 3 hours prior to the MTX infusion and continuing for 40 hours thereafter to ensure a large volume of alkaline urine.

Determination of MTX and Its Metabolites

Blood samples (0.5–1.0 ml) were collected at specified time intervals into heparinized tubes and plasma was chromatographed without further pre-cleanup procedure. Plasma samples not immediately analyzed were stored frozen at −16°C.

Urine samples were diluted 1:20 up to 1:2000 in distilled water and then samples of 50 μl were injected into the chromatographic system. Plasma and urine concentrations of MTX, 7-OH-MTX, and 2,4-diamino-

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[1] Received Nov 30, 1981; revised Mar 9, 1982; accepted May 25, 1982.

[2] Supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, FRG, grants Br 587/5 and Br 587/7.

[3] Medizinische Klinik der Justus Liebig-Universität, Giessen, Federal Republic of Germany.

[4] Institut für Transfusionsmedizin und Klinische Immunologie im Zentrum für Klinische Chemie, Klinische Immunologie und Humangenetik, Giessen, Federal Republic of Germany. E. Kuenzlen will present this work as part of his thesis to the Fachbereich Medizin der Justus Liebig-Universität.

[5] We thank Prof. Dr. H. M. von Hattingberg for evaluation of pharmacokinetic data, and Dr. H. Pralle for referral of patients and helpful discussions.

[*] *Reprint requests to:* Dr. H Breithaupt, Medizinische Klinik der Justus Liebig-Universitat, Klinikstrasse 36, D-6300 Giessen, Federal Republic of Germany.

N^{10}-methylpteroic acid (APA) were determined by HPLC using a Perisorb RP2-precolumn (3 × 0.4 cm, inside diameter) connected to a Spherisorb 5-μ ODS analytic column (15 × 0.4 cm) as stationary phase, and a ternary solvent mixture of citrate-phosphate (0.05 M, pH 3.2): methanol:tetrahydrofurane (80:16:4, by vol) as eluent. The pressure was maintained at 170 bar resulting in a flow rate of 2.0 ml/minute. Detection was set at 303 nm using a variable-wavelength photometer (Uvikon 720 LC, Kontron). MTX was eluted from the column at 3 minutes, 7-OH-MTX at 5.5 minutes, and APA at 8 minutes. The sensitivity of the assay system was 10^{-7} M for each substance, with a coefficient of variation < 7%. At 4×10^{-8} M the within-run coefficient of variation was 14.7% for MTX and 20.3% for 7-OH-MTX. Further details are published elsewhere (7).

Pharmacokinetic Calculations

Half-lives were calculated from plasma data by nonlinear curve fitting, based on the Gauss-Newton method with dynamic Hartley's modification (8). The computations were done by rotative iterative procedures on a Hewlett-Packard 9825 calculator. The total clearance (Cl_{total}) was obtained by dividing the total dose by the area under the curve (AUC) between the start of infusion and infinity. AUC was computed from the experimental data by parabolic interpolation and was extrapolated to infinity using the terminal slope. Extrapolation contributed < 1% to the MTX area and usually < 10% to the AUC of 7-OH-MTX. The total volume of distribution (V_{ss}) was obtained as the product of Cl_{total} and the mean transit time (T). T is computed as the area between the curve of cumulative disposition and its asymptote divided by this asymptote (8). The mean time of constant infusion, eg, 0.5 × infusion time, is subtracted from the mean system time to obtain the mean transit (or residence) time T for the drug in the body.

The fraction of MTX converted into 7-OH-MTX was not determined in this study. Volume terms are therefore not representative for the metabolite. From urinary data the renal clearances (Cl_{renal}) of MTX and 7-OH-MTX were evaluated. The values of V_{ss}, half-life, Cl_{total}, and Cl_{renal} are presented in table 1.

RESULTS

A typical time course of plasma concentrations of MTX and 7-OH-MTX during and after an infusion of high-dose MTX is shown in figure 1. Computer calculation of the plasma data indicates that elimination of MTX

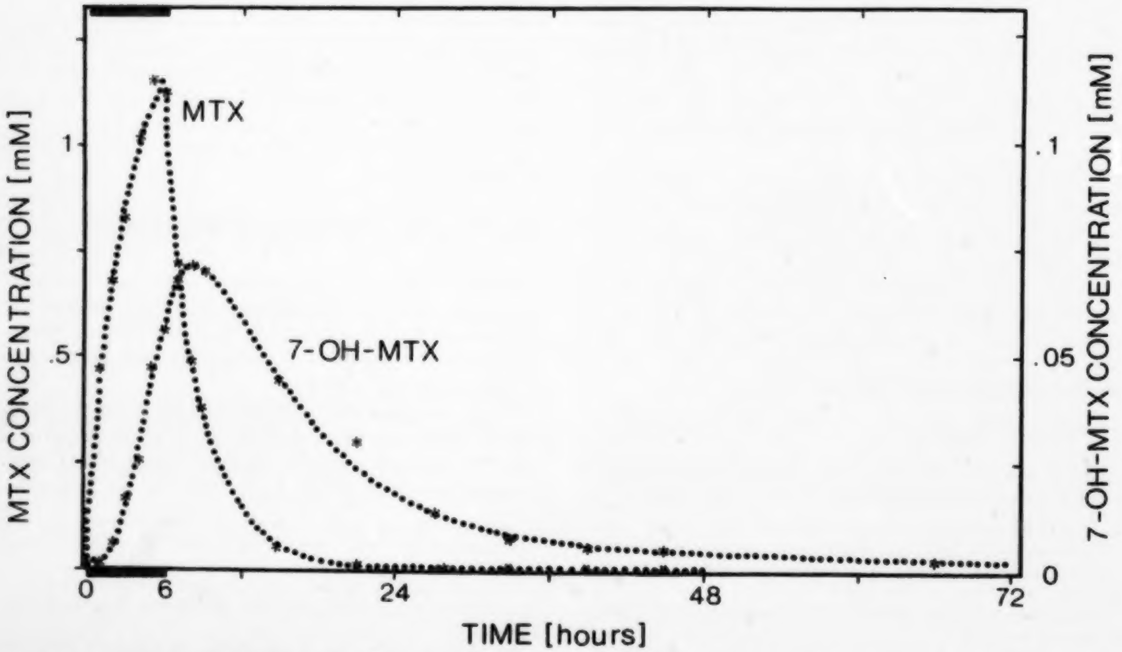

FIGURE 1.—Plasma kinetics of MTX and 7-OH-MTX during and after a continuous 6-hr infusion of 15 g of MTX (211 mg/kg; patient 4). The asterisks (*) represent observed values, whereas the points (●) were obtained by a computerized curve fitting.

TABLE 1.—Kinetic data of MTX and 7-OH-MTX following 6-hr infusions of high-dose MTX

Patient No.	Dose (mg/kg)	Kinetic data of MTX					Kinetic data of 7-OH-MTX		
		C_{max}* (μM)	V_{ss} (liter/kg)	β-half-life (hrs)	Cl_{total} (liter/kg/hr)	Cl_{renal} (liter/kg/hr)	C_{max} (μM)*	Half-life (hrs)	Cl_{renal} (liter/kg/hr)
1	204	1100	0.177	1.20	0.0778	0.0617	230	7.56	0.0137
2	200	580	0.179	2.75	0.1140	0.0769	100	11.0	0.0503
3	152	1100	0.142	2.13	0.0493	0.0376	290	22.2	0.0094
4	141	790	0.158	1.88	0.0547	0.0506	48	81.4	0.0155
	211	1100	0 177	2.29	0.0573	0.0534	71	40.8	0.0065
	282	1624	0.161	2.89	0.0467	0.0438	120	42.8	0.0118
5	137	620	0.191	2.11	0.0746	0.0677	65	37.5	0.0146
	205	1000	0.188	2.55	0.0599	0.0555	130	33.9	0.0096
	274	1700	0.156	3.51	0.0386	0.0372	110	31.6	0.0082
6	233	544	0.426	1.71	0.1660	0.1540	12	9.58	0.0269
	349	1124	0.269	2.19	0.0882	0.0839	118	11.0	0.0131
7	145	910	0.142	1.45	0.0569	0.0524	140	33.0	0.0077
	217†	1200	0.217	15.08	0.0392	0.0355	560	34.4	0.0050
Mean‡			0.186	2.08	0.0742	0.0623		23.8	0.0173
SE			0.062	0.58	0.0288	0.0232		15.2	0.0149

*Maximal concentration in plasma.

†Drug cycle followed by severe mucositis.

‡Mean of each patient was used for calculation (with exception of the toxic drug cycle of patient 7).

follows a two-compartment model, and elimination of 7-OH-MTX occurs mainly monoexponentially.

Plasma kinetics of MTX and 7-OH-MTX in a patient who received 6-hour infusions of 10, 15, and 5 × 20 *g* of MTX are shown in figure 2. The correlation between the peak plasma levels of MTX and 7-OH-MTX and the MTX dose applied was good. Records of five consecutive infusions of 20 *g* of MTX (282 mg/kg) revealed very similar plasma kinetics of MTX and 7-OH-MTX. The plasma values at indicated time intervals varied within a ± 1 SE range < 11%.

A scattergram of all MTX plasma values obtained from 122 high-dose infusions (fig 3a) shows that by 24–72 hours after the end of infusion most MTX plasma levels were below the toxic borderline reported to be 10^{-5} M at 24 hours, 10^{-6} M at 48 hours, and 10^{-7} M at 72 hours (5,9–14). Only one drug cycle was followed by plasma levels of MTX that were above this limit. Clinically a severe stomatitis occurred in this patient which subsided after 2 weeks. All other 121 high-dose MTX infusions had "nontoxic" MTX plasma kinetics, and there was no occurrence of severe toxic reactions except in one patient (No. 5) who repeatedly had serious hepatotoxic effects.

The plasma concentrations of 7-OH-MTX during and after 122 high-dose MTX infusions are shown in figure 3b.

The peak plasma levels of MTX were in the range of 540–1700 μM at the end of high-dose MTX infusion (table 1). Three hours after the end of infusion there were maximal plasma concentrations of 7-OH-MTX at 12–560 μM. Due to its slower disappearance from plasma, 7-OH-MTX exceeded the plasma concentrations of MTX at 3–10 hours following infusion of high-dose MTX.

When MTX plasma levels were in the range of 10^{-7} M, 7-OH-MTX was found to be 17–140 times higher than MTX.

The overall kinetic data obtained from seven patients treated with repeated drug cycles are listed in table 1. The total volume of distribution of MTX was 0.186 ± 0.062 liter/kg. The terminal β-half-life values of MTX were in the range of 2.08 ± 0.58 hours (mean ± SE), being about four times higher than the initial half-life values (0.58 ± 0.15 hours). Upon repeated treatment with high-dose MTX the intra-individual variation of the terminal half-life of MTX was < 7%, whereas its variation among patients was 28%. The mean time of MTX was 2.67 ± 0.61 hours, and the total clearance was found to be 0.072 ± 0.0288 liter/kg/hour. The renal clearance of MTX as calculated from urinary data was 0.0623 ± 0.0232 liter/kg/hour, indicating a metabolic (or nonrenal) contribution of about 16% to the total clearance of MTX.

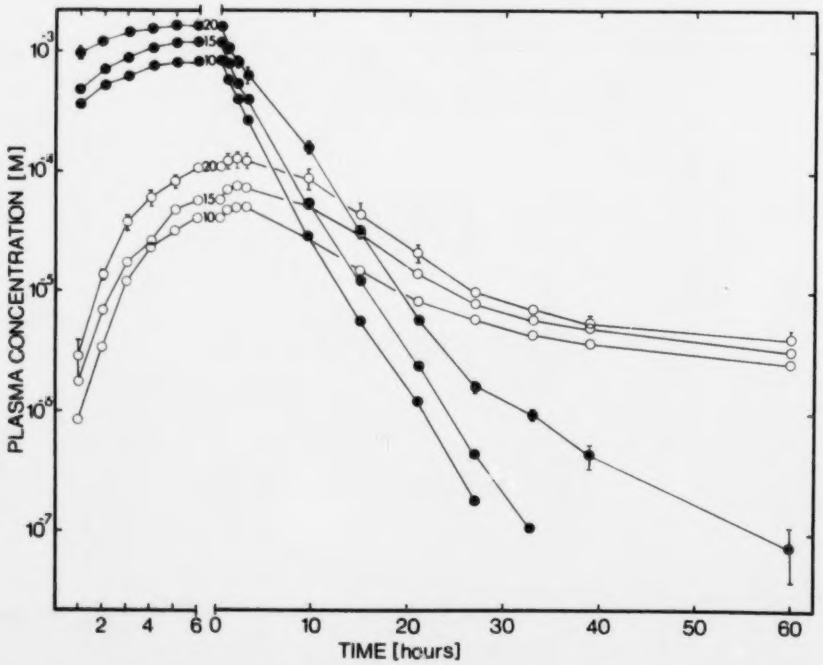

FIGURE 2.—Plasma concentrations of MTX (●) and 7-OH-MTX (○) in 1 patient (No. 4) treated with repeated 6-hr infusions of high-dose MTX (1 × 10 g ≙ 141 mg/kg; 1 × 15 g ≙ 211 mg/kg; 5 × 20 g ≙ 282 mg/kg). 20-g infusions were applied at 4-wk intervals, and their plasma values are shown as mean ± 1 SE.

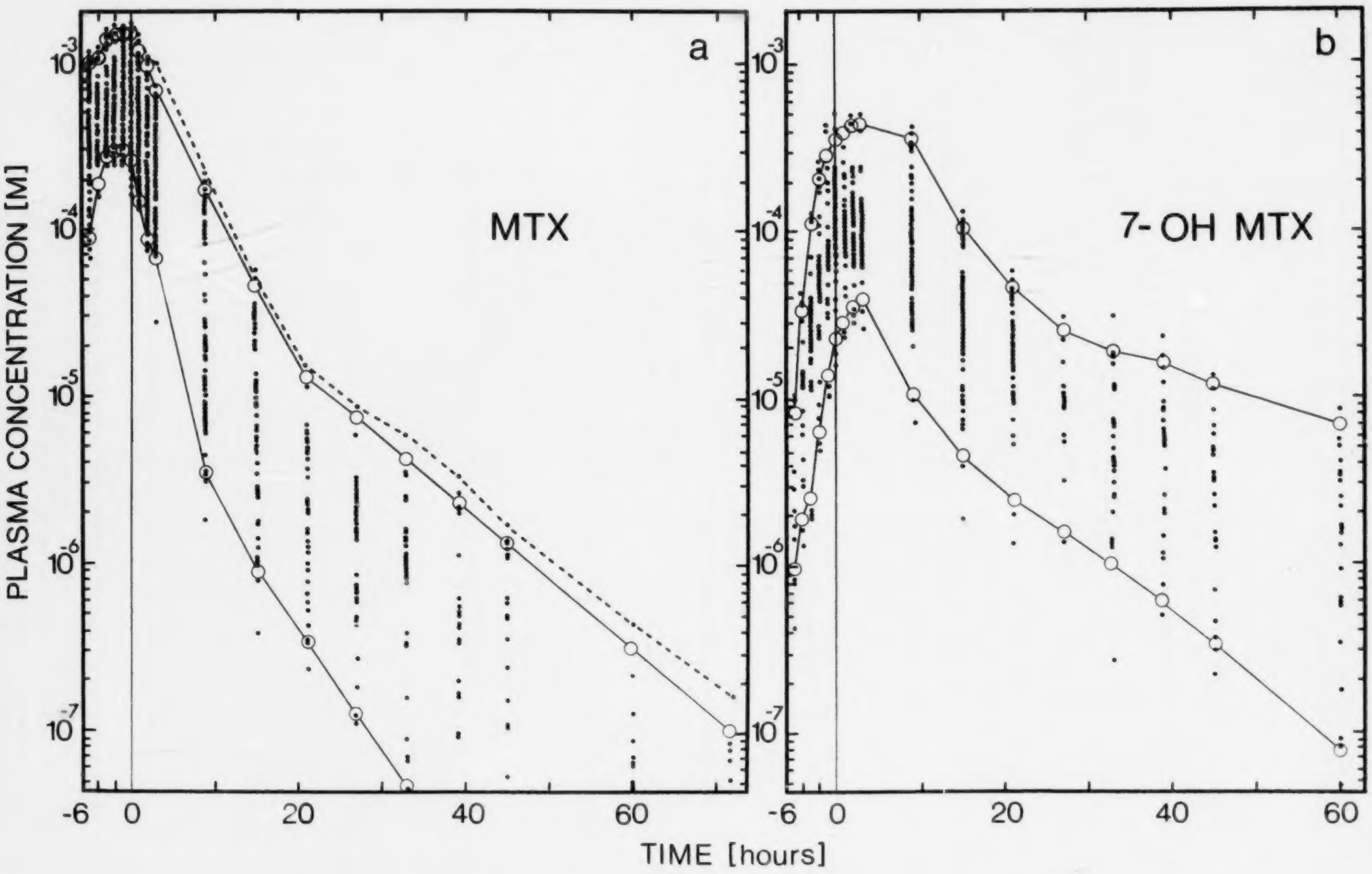

FIGURE 3.—Scattergrams of plasma concentrations of MTX (a) and 7-OH-MTX (b) following 122 6-hr MTX infusions of 140–350 mg/kg. Dotted line shows the MTX plasma kinetics of a drug cycle which was followed by severe mucositis (patient 7). Solid lines represent the 5 and 95 percentiles.

In contrast to the elimination of MTX, the plasma disappearance of 7-OH-MTX was mainly monoexponential, with half-life values of 23.8 ± 15.2 hours (mean ± SE). The renal clearance of 7-OH-MTX was 0.0173 ± 0.0149 liter/kg/hour.

The cumulative urinary excretion of MTX and 7-OH-MTX as monitored over a period of 120 hours is presented in figure 4. About 50% of the dose of MTX was excreted within the first 12 hours after the start of infusion, and about 80% was recovered within the first day. As shown in table 2, recovery of MTX ranged from 70.2% to 94.0% (mean, 85.4), whereas 0.4%–11.1% (mean, 3.8) was excreted as 7-OH-MTX. The recovery of MTX and 7-OH-MTX was quite reproducible intra-individually on repeated application of high-dose MTX.

The MTX metabolite APA was found in the urine in small amounts of up to 0.01% of the applied dose. In some plasma samples APA was demonstrable up to a concentration of 10^{-7} M.

Some evidence for biliary secretion and enterohepatic recirculation of MTX and 7-OH-MTX is provided by the following data: First, a considerable amount of MTX (480 mg) and 7-OH-MTX (63 mg) was found in the gastric juice vomited at the end of a high-dose MTX infusion. The gastric juice was contaminated by some bilirubin glucuronide (1.9 mg/100 ml). Second, the plasma disappearance of MTX and 7-OH-MTX was influenced by coadministration of activated charcoal, which was given orally for 3 days beginning 12 hours before the start of the high-dose MTX infusions (fig 5). Following daily administration of 10 g of activated charcoal, the plasma half-life of MTX was 84% and the half-life of 7-OH-MTX was 44% of the controls, whereas the total clearance of MTX increased by 112%. During the drug cycles with coadministered charcoal the urinary recovery decreased by 11% with respect to MTX and by 71% for 7-OH-MTX.

DISCUSSION

Previous data on the kinetics of high-dose MTX were based on assays that could not differentiate the metabolites (5,13,15–29), or were influenced by immunologic cross-reactions (14,30). The cross-reactivity of 7-OH-MTX known to be in the range of 1%–10% (31–34) is relevant for the determination of MTX in the final phase of disappearance from plasma at 48–72 hours, since 7-OH-MTX levels were shown to be up to 140 times higher than those of MTX. The cross-reactivity of the MTX metabolite APA, which was occasionally detected in plasma and urine (7), has been reported to be 100% in the immunologic assays (31–35). Therefore, the accuracy of the test methods used in previous kinetic studies remains questionable, in particular when applied to plasma samples collected in the terminal phase of MTX elimination.

The discrepancy between the terminal half-life value of MTX in this study and the half-life values of 5.6–16 hours reported previously (19,21,23,27,29) for the post-infusion period from 15 to 60 hours may be caused by

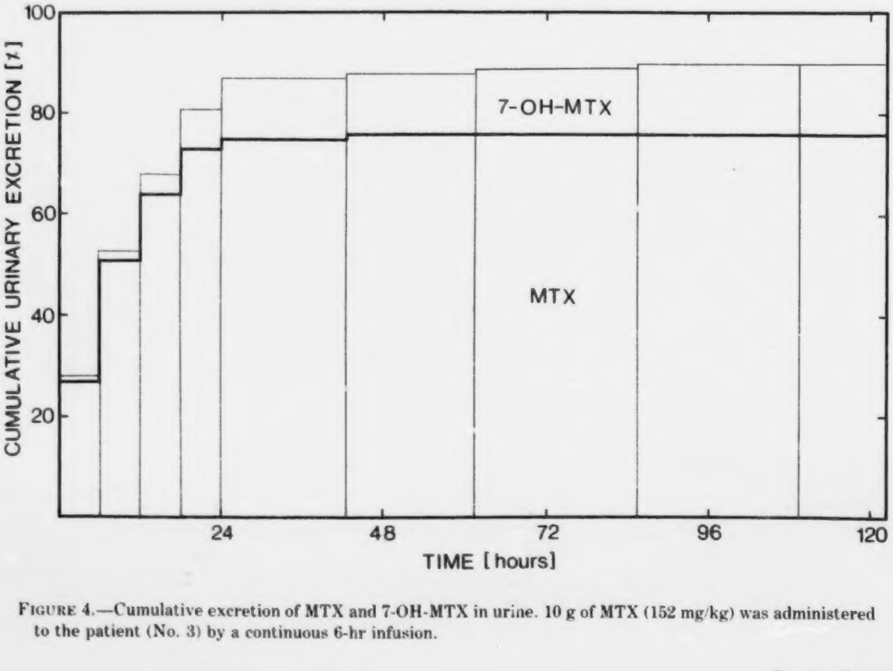

FIGURE 4.—Cumulative excretion of MTX and 7-OH-MTX in urine. 10 g of MTX (152 mg/kg) was administered to the patient (No. 3) by a continuous 6-hr infusion.

TABLE 2.—Urinary recovery following treatment with high-dose MTX.

Patient No.	Dose (g)	Drug cycles	Recovery of MTX (%)		Recovery of 7-OH-MTX (%)	
			Mean	SE	Mean	SE
1	10	6	78.7	12.5	3.9	2.2
2	10	9	70.2	16.9	3.5	2.3
3	10	12	75.7	7.5	11.1	3.8
4	10–20	12	93.2	7.8	2.7	1.1
5	10–20	4	93.8	8.8	3.6	1.0
6	10–15	4	92.2	7.8	1.7	0.4
7	10–15	3	94.0	3.5	0.4	0.2
Mean			85.4		3.8	

interferences with 7-OH-MTX and APA in the previous assay methods. However, a recent comparison of several different assay methods including HPLC did not support this assumption (34). The initial phase of MTX disappearance from plasma (α-half-life) was approximately four times shorter than the β-half-life of MTX. A similar value for the α-half-life (0.75 hours) was reported for conventional doses of MTX (16).

The volume of distribution was about 18% of body weight, which approximates the extracellular space. A much greater value of 75% was reported for conventional doses of MTX (36). MTX is not tightly bound to plasma proteins. We reported a plasma protein binding of 53% ± 8% for MTX levels from 10^{-6} to 10^{-3} M (7). In previous reports MTX plasma protein binding ranged from 20% to 96% (9). In ultrafiltration experiments (7) protein binding of APA was similar to MTX (56% ± 8%), whereas 7-OH-MTX bound to plasma proteins to a greater degree (93% ± 2%).

The plasma clearance of MTX following conventional doses was reported to be in the range of 80–160 ml/minute (23–25), which favorably compares with the MTX clearance reported in this study. In contrast to the assumption of others (15), escalation of dose up to 350 mg/kg was followed by a linear increase of MTX levels in plasma.

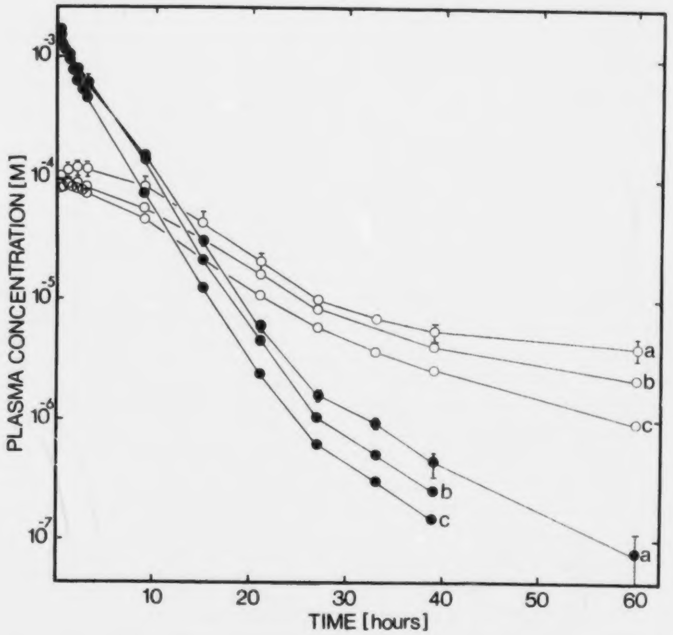

FIGURE 5.—Influence of oral application of activated charcoal on plasma kinetics of high-dose MTX. Disappearance curves of MTX (●) and 7-OH-MTX (○) are shown (patient 4). (a) Mean ± SE of 5 infusions without coadministration of activated charcoal; (b) daily application of 5 g of activated charcoal, beginning 12 hrs before MTX infusion and continuing for 3 days; and (c) daily application of 10 g of activated charcoal.

The renal clearance of MTX was approximately 84% of the total clearance of MTX, thus indicating that MTX is mainly eliminated by the kidneys, presumably by glomerular filtration and to a lesser degree by tubular secretion as suggested by others (37). The renal clearance of MTX, being about 65% of the glomerular filtration rate, was comparable to clearance values previously published for high-dose MTX (23,24) and for low-dose MTX (37,38). The constant renal clearance of MTX at plasma levels, which ranged from 10^{-4} to 10^{-7} M, supports the evidence for dose-independent elimination of MTX (up to 350 mg/kg). The cumulative urinary recovery of MTX and 7-OH-MTX in this study agrees well with the findings of others (18,39) but is inconsistent with a recovery $< 60\%$ reported in another study (24).

To our knowledge few data have been published regarding the kinetics of 7-OH-MTX (40). 7-OH-MTX peaked 3 hours after infusion of high-dose MTX (range, 12–560 μM). The plasma disappearance of 7-OH-MTX was mainly monoexponential, with a half-life about 11 times longer than the terminal half-life of the parent compound MTX. The renal clearance of 7-OH-MTX was four times smaller than the renal clearance of MTX. The urinary recovery of 7-OH-MTX indicates a significant elimination of MTX by metabolism. Similar recoveries of 7-OH-MTX were reported by other investigators (23,41), who suggested that 7-OH-MTX may contribute to the renal toxicity of high-dose MTX in man, since precipitation of the less soluble 7-OH-MTX in the renal tubules has been demonstrated to occur in monkeys (42). The large variability of 7-OH-MTX formation among patients is in contrast to the highly reproducible metabolic formation in one single patient. Repeated infusions of high-dose MTX do not provide for the assumption that hydroxylation of MTX at position 7 is an inducible metabolic step.

During and following high-dose infusions of MTX a considerable proportion of the applied dose was demonstrated to occur in the vomit, which was contaminated by bile. From our data we conclude that biliary levels of MTX and 7-OH-MTX are about 200 times higher than simultaneous plasma levels. This appears to indicate that enterohepatic circulation of MTX and its 7-hydroxy metabolite may be relevant. The excretion into the bile is a controversial issue in current literature (23,25,43,44). The enterohepatic circulation was emphasized by the influence of severe vomiting and diarrhea on plasma clearance and urinary elimination of MTX (45). Our data on the interference of coadministered activated charcoal with enterobiliary recirculation of MTX and 7-OH-MTX and its effects on plasma and renal clearances are preliminary. Further studies of biliary excretion of MTX and 7-OH-MTX in patients receiving high-dose MTX are in progress.

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Dose-Response Curves for Predicting Misonidazole-Induced Peripheral Neuropathy[1]

J. G. Schwade, R. W. Makuch, J. M. Strong, and E. Glatstein[2,*]

Using pharmacokinetics determined for 27 patients who received iv misonidazole, we have studied the relationship between various pharmacokinetic parameters and the probability of observing peripheral neuropathy. By plotting the probability of observing peripheral neuropathy as a function of each individual parameter and using a linear logistic regression model, we have found a general sigmoid response relating the observations of peripheral neuropathy to the area under the pharmacokinetic curve (AUC), the total cumulative dose throughout the course, the plasma concentration, the plasma elimination half-life, and the total cumulative AUC. By using linear logistic regression analysis, the best correlation with observing peripheral neuropathy was seen with the AUC. Statistical considerations are discussed in depth. This type of analysis may well prove useful for other toxic agents used in cancer treatment. [Cancer Treat Rep 66:1743–1750, 1982]

Recent interest in the effort to increase the susceptibility of tumors to radiation has centered on sensitizers of hypoxic cell populations. The nitroimidazoles, primarily the 5-nitroimidazole metronidazole (Flagyl) and the 2-nitroimidazole misonidazole (Ro 7-0582), have been the most promising and most studied of available compounds.

Although significant selective sensitization of hypoxic cells can be obtained both in vitro and in vivo (1,2), efficacy in patients has been demonstrated only with metronidazole and only in one study, with enhanced median survival but no cure (3). Since in vitro and in vivo studies indicate more effective sensitization with misonidazole, that drug has been selected for more intensive study of hypoxic cell radiosensitization in patients (2).

While nausea and emesis may frequently occur (3–6), peripheral neuropathy is the major dose-limiting toxic effect of misonidazole (4–7). Attempts have been made to correlate this toxicity with various pharmacologic parameters, including the total dose (4,6) and the area under the curve (AUC) in which misonidazole plasma levels are plotted as a function of time (4).

We have determined certain misonidazole pharmacologic parameters in patients after iv administration of the drug and have applied a unique statistical analysis to determine whether any correlation exists between these measurable parameters and the observed neurotoxicity. Our findings indicate that AUC shows the best correlation and should be of value in identifying those individuals at greatest risk for peripheral neuropathy after misonidazole administration.

MATERIALS AND METHODS

Plasma pharmacokinetics were determined in 27 patients who received misonidazole. Formulated drug was provided by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), lyophilized in 500-mg amber vials, and reconstituted with 19.5 ml of normal saline. Infusion was carefully timed over 5 minutes. The drug was infused into a vein in an upper extremity, and at least three plasma samples were obtained in 1–24 hours following the start of infusion. Misonidazole was administered in doses of 1.5 or 2.0 g/m^2 twice weekly for five to 14 doses (tables 1 and 2). Toxicity consisted of sensory peripheral neuropathy (13 patients), nausea and/or emesis (eight), and tinnitus (two). Toxicity was mild and self-limited in all but two patients, who were hospitalized because of severe peripheral neuropathy (table 2).

Plasma levels of misonidazole and desmethylmisonidazole were determined by high-pressure liquid chromatography, using a modification of the method described by Workman et al (8). Patients received misonidazole either as part of a phase I trial or as part of an ongoing randomized clinical trial testing the efficacy of the drug with irradiation in patients with carcinoma of the esophagus or locally unresectable osteosarcoma. All patients signed informed consent forms.

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[1] Received Dec 18, 1981; revised May 28, 1982; accepted June 30, 1982.

[2] Radiation Oncology Branch (*J. G. Schwade* and *E. Glatstein*), Biometric Research Branch (*R. W. Makuch*), and the Laboratory of Chemical Pharmacology (*J. M. Strong*), Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Eli Glatstein, MD, Radiation Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm B3B38, Bethesda, MD 20205.

TABLE 1.—Occurrence of peripheral neuropathy in patients who received misonidazole iv twice/wk*

Patient No.	n	C_0 (μg/ml)	CV (%)	$T_{1/2}$ (hrs)	CV (%)	AUC (μg·hr/ml)	CV (%)	Dose/ fraction (g)	Total dose (g)	No. of doses	Total AUC (μg·hr/ml)
						1.5 mg/m²					
1	1	63.3		10.9		1000		2.55	25.5	10	10,000
2	1	82.5		8.9		1060		2.3	11.5	5	5300
3	6	72.8	14	8.7	6	912	13	3.0	30.0	10	9120
4	1	62.3		15.3		1375		2.7	27.0	10	13,750
5	1	58.2		8.1		677		2.25	11.25	5	3385
6	1	58.7		7.4		735		2.7	27.0	10	7350
7	2	63.1	11	8.6	7	781	5	2.5	25.0	10	7810
8	1	68.8		7.4		735		2.6	26.0	10	7350
9	5	56.0	12	9.3	1	753	13	2.7	27.0	10	7530
10	1	86.2		8.9		1107		2.3	11.5	5	5535
11	4	74.9	3	5.2		566	10	2.5	25.0	10	5660
12	1	60.6		8.8		770		3.0	15.0	5	3850
Median		63.2		8.8		776		2.58	25.5	10	7350
Range		56.0–86.2		5.2–15.1		566–1375		2.25–3.0	11.3–30.0	5–10	3385–13,570
						2.0 mg/m²					
13	1	72.4		10.7		1118		3.5	17.5	5	5590
14	1	59.0		8.2		698		2.5	12.5	5	3490
15	4	90.1	28	9.0	9	1150	19	3.25	22.8	7	8050
Median		72.9		9.0		1118		3.25	17.5	5	5590

*CV = coefficient of variation, n = No. of determinations.

TABLE 2.—Absence of peripheral neuropathy in patients who received misonidazole iv twice/wk*

Patient No.	n	C_0 (μg/ml)	CV (%)	$T_{1/2}$ (hrs)	CV (%)	AUC (μg·hr/ml)	CV (%)	Dose fraction (g)	Total dose (g)	No. of doses	Total AUC (μg·hr/ml)
						1.5 mg/m²					
16	1	58.8		8.8		745		2.6	15.6	6	4470
17	1	87.2		8.4		1060		3.6	27.6	7	7420
18	1	61.3		16.3		1442		2.8	22.4	8	11,536
19	8	63.4	8	9.5	50	917	43	3.3	42.9	13	11,921
20	1	75.7		14.6		1591		3.46	24.2	7	11,137
21	8	70.8	12	10.1	8	1024	6	2.8	28.0	10	10,240
22	4	68.2	5	10.3	14	1012	9	3.0	30.0	10	10,120
23	2	80.0	15	11.0	7	1259	8	2.6	20.8	8	10,072
Median		69.5		10.2		1042		2.9	25.9	8	10,180
Range		58.8–87.2		8.4–16.3		745–1591		2.6–3.6	15.6–42.9	6–13	4470–11,921
						2.0 mg/m²					
24†								2.4	16.8	7	
25	3	123	16	9.5	17	1718	28	3.25	22.8	7	12,026
26†								2.9	26.1	9	
27	4	104	17	14.1	6	2121	13	3.6	25.2	7	14,847
28	1	92		12.3		1644		3.5	35.0	10	16,440
29	1	79.6		10.3		1180		4.0	32.0	8	9440
Median		98		11.3		1681		3.39	25.7	7.5	13,437
Range		77.6–123		9.5–14.1		1180–2121		2.4–4.0	16.8–35.0	7–10	9440–16,440

*CV = coefficient of variation, n = No. of determinations.

†Patient excluded from all calculations.

Statistical Methods

For each parameter examined, the median and range were determined within the patient groupings defined by the dose of misonidazole (1.5 g/m^2 or 2.0 g/m^2) and by whether a peripheral neuropathy was present. A blocked Wilcoxon rank-sum test adjusted for ties, with the dose of misonidazole as the blocking factor, was used to compare the value of each parameter between patients who developed or did not develop peripheral neuropathy. The normal approximation was used to calculate two-sided significance probabilities (9).

A linear logistic regression model and maximum likelihood techniques (10) were used to characterize the relationship between each parameter and the probability of having peripheral neuropathy. The actual dose of misonidazole was included as a covariate in each regression analysis. Statistical significance of the covariate was evaluated in each analysis, using standard normal theory to determine whether it was significantly different from 0. If it was not, then a simpler expression resulted, with only one independent variable. One consequence is that the relation between each parameter and the probability of developing peripheral neuropathy can be illustrated graphically. A goodness-of-fit test for each logistic regression model (11) was computed to ensure that the data were described adequately by the model. Using the patient's observed parameter value, the fitted regression model was then employed to classify a patient into one population (peripheral neuropathy) or another (no peripheral neuropathy). Using the fitted regression model, perfect patient discrimination occurs when each patient is correctly predicted to have or not to have peripheral neuropathy. Such perfect prediction is unexpected for a number of reasons, including patient heterogeneity. To quantify the extent of departure from perfect discrimination, a misclassification error probability was calculated. It is defined as the ratio of the number of patients who were classified incorrectly using the regression model and the total number of patients evaluated. Since this naive projection tends to underestimate the true misclassification error rate, the "leave-one-out jackknife" (12) was used to provide less-biased error probabilities.

Pharmacokinetic Analysis

The pharmacokinetic parameters used in this analysis were determined after obtaining at least three blood samples drawn during the 1-24-hour interval after iv administration of misonidazole. A least squares analysis was used to fit the data to a single compartment.

Pharmacokinetic Model

The curve plotting misonidazole plasma levels as a function of time was best described by a biexponential function following a 5-minute iv injection. However, as previously reported (5), the initial distribution phase had a mean half-life of 5.8 minutes. Therefore, for the purposes of this study, only the elimination phase was evaluated, since the pharmacokinetic parameters considered in this report would not be significantly affected by the early distribution phase. All parameters were calculated from the misonidazole plasma levels using the relationship $C_t = C_0 e^{-\beta t}$ (equation 1), where C_t(μg/ml) is the misonidazole plasma level at time t (minutes), C_0 (μg/ml) is the apparent plasma misonidazole concentration at time equal to 0, and β (hour^{-1}) is the slope of the best-fit line to data based on equation 1 (fig 1). The misonidazole plasma elimination half-life $T_{1/2}$ (hour) was determined from the relationship: $T_{1/2} = \ln 2/\beta$ (equation 2). An estimate of the area under the plasma misonidazole concentration-time curve (AUC) was made using the following equation: AUC $= C_0/\beta$ (equation 3).

When the AUC is determined using equation 3, plasma misonidazole levels obtained before 1 hour after drug administration should not be included in the calculation. This is to ensure that the drug has reached equilibrium between the plasma and its major body tissue compartments.

Alternate methods for determining AUC, such as the trapezoidal rule (13), may be applied, especially when the drug is administered orally. However, a much larger number of misonidazole plasma level determinations

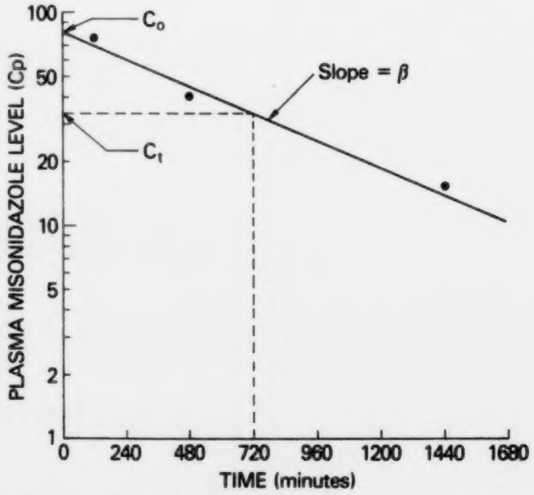

FIGURE 1.—Pharmacokinetic plot of plasma misonidazole levels as function of time (*see* text).

would be required to obtain a good estimate of the AUC, following administration by the oral route.

While multiple AUC determinations were obtained for one patient, a "total AUC" was determined in other patients by multiplying the mean AUC by the number of administered doses. Thus, for most patients, total AUC represents an approximation. Detailed pharmacokinetics, including levels in plasma, pleural fluid, and tumor, have previously been reported in selected patients (5).

RESULTS

Values of four pharmacologic parameters, C_0, $T_{1/2}$, AUC, and total AUC, were compared between patients who developed or did not develop peripheral neuropathy. Those with peripheral neuropathy tended to have higher C_0 than patients without peripheral neuropathy ($P = 0.08$). The values for the remaining three parameters were significantly higher in patients with peripheral neuropathy ($P = 0.013$, 0.005, and 0.004, respectively). Similar analyses were performed to compare the two groups for total dose and the number of given doses. Total dose tended to be higher in patients with peripheral neuropathy compared with those without peripheral neuropathy ($P = 0.066$). The number of doses given was not significantly associated with the development of peripheral neuropathy ($P = 0.27$).

The association between each of the above parameters and the probability of peripheral neuropathy was determined using the linear logistic model with misonidazole in g/m^2 as a covariate. To carry out the analysis, patients were divided into one of two groups. For patients who developed peripheral neuropathy, a value of 1 was assigned as the dependent variable; patients who did not develop peripheral neuropathy were assigned a value of 0. A clear dose-response relationship was found between AUC and the probability of developing peripheral neuropathy (fig 2). The covariate, dose, was excluded from the equation since it was not significantly different from 0 ($P = 0.47$). The parameter AUC was significantly different from 0 ($P = 0.016$). Similar findings were also evident for $T_{1/2}$ (fig 3), total AUC (fig 4), and C_0 (fig 5). However, the covariate was of some importance ($P = 0.15$) in the regression equation involving total dose, and the two parameters were highly correlated with one another. Thus, the parameter total dose was eliminated from further consideration since a single graphic display and any clear interpretation of the parameter were complicated by the necessary inclusion of the covariate in the regression model.

The fitted regression model for each parameter is indicated below each figure. The equation can be used to predict whether a patient with a specified parameter value, X_* (eg, the patient's AUC), will develop peripheral neuropathy. Specifically, the parameter value, X_*, is inserted in the equation $\hat{\alpha} + \hat{\beta}X$, and if the resultant quantity is ≤ 0, then the probability of the occurrence of peripheral neuropathy predicted from the model is ≤ 0.50. Since patients without peripheral neuropathy were assigned initially a value of 0 for the dependent variable in the regression model, a reasonable classification rule is that the model predicts that a patient with parameter value of X_* will not develop peripheral neuropathy if $\hat{\alpha} + \hat{\beta}X_* \leq 0$. Conversely, if the quantity $\hat{\alpha} + \hat{\beta}X_* > 0$, then the model predicts that peripheral neuropathy will develop in the patient.

To illustrate, suppose the parameter AUC is used to predict whether patients will or will not develop peripheral neuropathy. The equation is $y = \hat{\alpha} + \hat{\beta}X =$

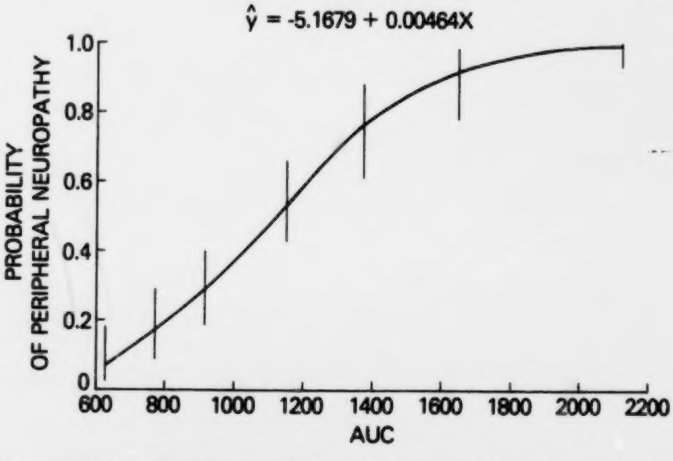

FIGURE 2.—Dose-response curve, using linear logistic model; shows probability of developing peripheral neuropathy as function of area under pharmacokinetic curve. Error bars represent ± 1 SE.

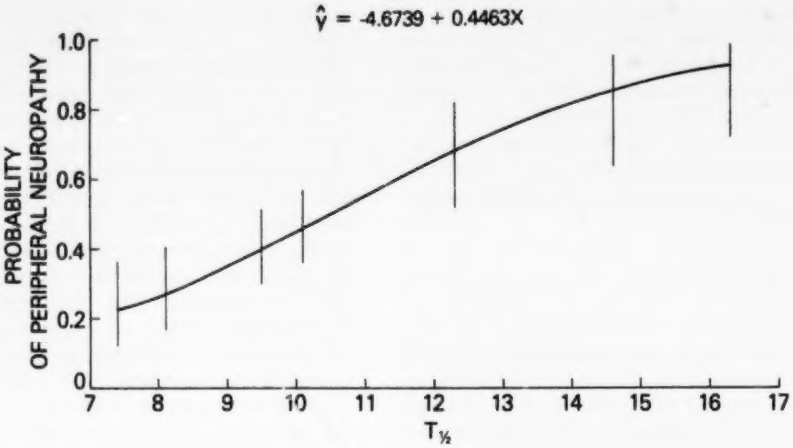

FIGURE 3.—Dose-response curve using linear logistic model for probability of developing peripheral neuropathy as function of half-life. Error bars represent ± 1 SE.

$-5.1679 + 0.00464X$ (fig 2). Then $y \leq 0$ whenever $X \leq 1113$. This implies that the model is used to predict that a patient will not have peripheral neuropathy if the AUC is ≤ 1113. The data in table 1 indicate that the model's prediction is incorrect for patients 4, 13, and 15, since their values exceed 1113 and yet it is known that they did not have peripheral neuropathy. Likewise, the model predicts that a patient will have peripheral neuropathy if the AUC exceeds 1113. Table 2 shows that the model incorrectly predicted the status of patients 16, 17, 19, 21, and 22.

The sum of these two types of misclassification divided by the total number of patients provides a misclassification error rate estimate of $(3 + 5)/27 = 29.6\%$ if AUC is used. Error rate estimates are calculated in a similar manner for the other parameters; they are 37.0%, 14.8%, and 33.3% for $T_{1/2}$, total AUC, and C_0, respectively. These estimates of the true misclassification error rate may be too low since the same data were used to obtain the fitted regression model. For this reason the jackknife method, a cross-validation technique, was used to obtain less-biased error rate

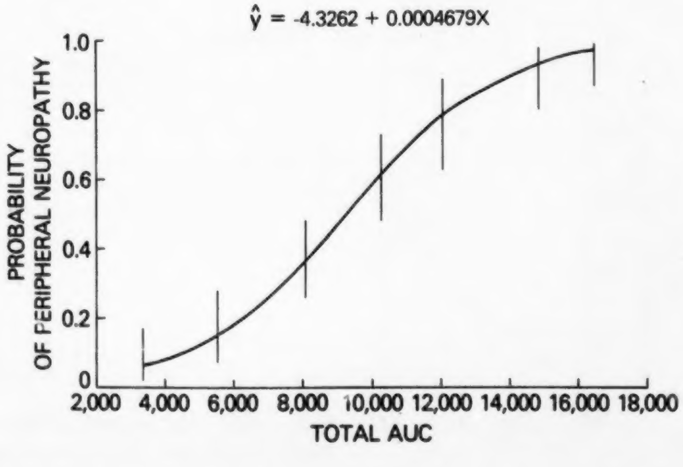

FIGURE 4.—Dose-response curve, using linear logistic model for probability of developing peripheral neuropathy as function of total area under pharmacokinetic curve (AUC × total No. of injections). Error bars represent ± 1 SE.

estimates. This method gives error rate estimates of 33.3%, 37.0%, 22.2%, and 33.3% for AUC, $T_{1/2}$, total AUC, and C_0, respectively.

Goodness-of-fit tests indicated that the final regression model for each parameter summarized the observed data quite well since the two-sided *P* values exceeded 0.40 in each instance. Although fit is one criterion for evaluating a risk function, it is also desirable for the risk slope to be steep. A steep slope indicates that the patients are classified correctly by the model with a high degree of success. Visual inspection of figures 2–5 suggests that AUC and total AUC are the best candidates for discriminating effectively in regard to the frequency of peripheral neuropathy.

DISCUSSION

The clinical utility of hypoxic cell radiosensitizers is still unproved. To evaluate these compounds effectively, maximally tolerated plasma levels should be obtained to achieve optimal concentrations of sensitizers in the region of hypoxic cells at the time of irradiation. Additionally, in patients with resistant strains of *Trichomonas vaginalis*, which is relatively resistant to metronidazole, use of adequate doses of misonidazole may require knowledge of individual susceptibility to toxicity (14,15). While some authors support a strong correlation between neurotoxicity and total dose (4,6), our results and those of Walker and Strike (7) do not support this finding. At best, total dose is only a crude indicator of pharmacokinetic parameters, mainly AUC, $T_{1/2}$, and peak plasma level (C_0 in this case). While total dose may be useful in obtaining information to determine starting doses for a course of therapy, it has little value in predicting toxic effects for individual patients. Determination of pharmacokinetic parameters such as AUC, $T_{1/2}$, and C_0 does allow identification of an individual's probability of developing peripheral neuropathy. AUC, $T_{1/2}$, and C_0 can easily be determined from three or more plasma levels obtained 1–24 hours after administration of the initial dose and at intervals during the course of therapy. A total AUC can be estimated by multiplying AUC by the number of planned doses, which gives an estimate of the probability of peripheral neuropathy with the planned dose. Appropriate adjustments in dose can thus be made or courses of therapy can be lengthened or abbreviated, in accordance with dose-response curves. While this paper contains dose-response data only for misonidazole toxicity, similar information might be useful for other drugs used in cancer therapy, particularly cytotoxic agents that are thought to have dose-response relationships for tumor response and that undoubtedly have individual variation in therapeutic ratio.

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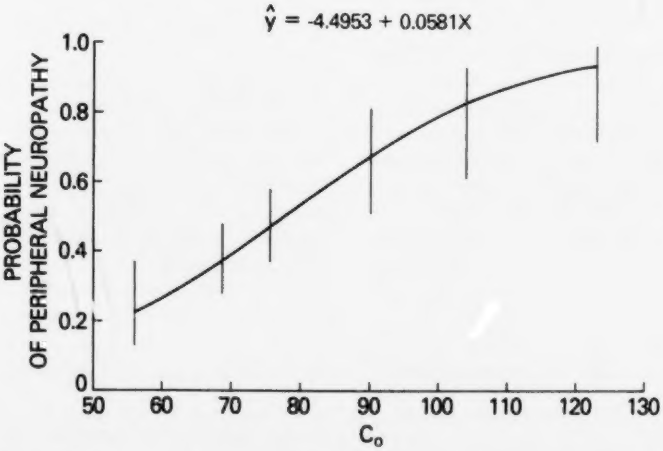

Figure 5.—Dose-response curve, using linear logistic model for probability of developing peripheral neuropathy as function of apparent plasma concentration at time = 0 (*see* text). Error bars represent ± 1 SE.

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Metabolism and Mechanism of the Antileukemic Action of Isomeric Aryldimethyltriazenes[1,2]

G. Sava,* T. Giraldi, L. Lassiani, and C. Nisi[3,4]

Mice bearing TLX5 lymphoma or P388 leukemia were treated with *ortho*, *meta*, and *para* isomers of the salts of (3,3-dimethyl-1-triazeno)benzoic acid. Survival time was markedly increased in mice given the *para* isomer; effects were less pronounced for the *meta* isomer and absent for the *ortho* isomer. The in vivo effects of the tested compounds did not correlate either with the propensity of the drugs to undergo oxidative *N*-demethylation and hydrolysis to diazonium cations or with in vitro cytotoxicity for TLX5 lymphoma cells. The *para* isomer did not reduce the number and viability of peritoneal TLX5 lymphoma cells after in vivo and in vitro treatment, whereas a dose-dependent reduction that can even result in the absence of clonogenic tumor cells occurred in the brains of the treated animals. These data indicate that the increased survival time of the tumor-bearing mice treated with the *para* isomer should not be ascribed to cytotoxic effects of the drug and might be attributed to inhibition of tumor cell dissemination in various organs of the host, as already observed for solid metastasizing tumors in mice. [Cancer Treat Rep 66:1751–1755, 1982]

The imidazole dimethyltriazene dacarbazine displays antitumor activity against several rodent transplantable tumors (1) and is also usefully employed in clinical practice as a single drug or integrated into combined modalities for the treatment of malignant melanoma and other tumors in man (2–4). The structurally related aryldimethyltriazenes also display antitumor activity in experimental systems, in some instances equal to or greater than that displayed by dacarbazine (5–7). Evidence has been provided in experimental systems that dimethyltriazenes require metabolic activation to exert their antitumor cytotoxic activity (5). Monomethyltriazenes produced via the formation of 3-hydroxy-3-methyltriazene by microsomal oxidative *N*-demethylation of the parent dimethyl compounds have been suggested as being the active metabolites (8–10). Yet, examination of the measurements performed on in vitro demethylation and in vivo antitumor activity for several dimethyltriazenes (11,12) shows no apparent correlation between them. A possible active role of the diazonium cations derived by hydrolysis has been ruled out, since dimethyltriazenes stable in aqueous solution for the length of the experiments have good antitumor activity (5) and since no antitumor effects result from the in vivo administration of aryldiazonium cations (5,13).

Aryldimethyltriazenes having different substituents on the benzene ring have mainly been used so far in investigating the structure-activity relationships of this group of drugs. In the present investigation the *ortho* (*o*-DM-COOK), *meta* (*m*-DM-COONa), and *para* (*p*-DM-COOK) isomers of the salts of (3,3-dimethyl-1-triazeno)benzoic acid, having different steric properties but closely similar lipophilicity, have been examined. *o*-DM-COOK and *m*-DM-COONa are isomeric derivatives of *p*-DM-COOK, which has already been found to prolong the survival time of leukemic mice (14) and to markedly suppress the metastatic spread of solid mouse tumors (13,15). The in vitro cytotoxicity for TLX5 lymphoma cells has thus been compared with the in vivo increase of survival time of mice implanted ip with the same tumor or with P388 leukemia. Furthermore, the half-life for hydrolysis to diazonium cations in aqueous solution at neutral pH and the oxidative *N*-demethylation by 10,000 x *g* rat liver supernatant for the three compounds used have been determined in order to examine possible relationships between these parameters and antitumor effects. Finally, the effects of the treatment with *p*-DM-COOK on the growth and viability of peritoneal tumor cells, as well as on the presence of clonogenic tumor cells in the brains of TLX5 lymphoma-bearing mice, have been determined.

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[1] Received Dec 15, 1981; revised May 18, 1982; accepted June 16, 1982.

[2] Supported by Italian National Research Council—Special Project "Control of Neoplastic Growth" (contract 80.01562.96) and by a special grant from the University of Trieste.

[3] Istituto di Farmacologia (*G. Sava* and *T. Giraldi*) and Istituto di Chimica Farmaceutica (*L. Lassiani* and *C. Nisi*), Università di Trieste, Italy.

[4] We acknowledge the skillful technical assistance of Dr. Rinalda Campanini.

**Reprint requests to:* Dr. Gianni Sava, Università degli Studi di Trieste, Istituto di Farmacologia, Via A. Valerio, N. 32, I-34100 Trieste, Italy.

MATERIALS AND METHODS

The synthesis of the compounds used was performed following already reported procedures (16).

In Vitro Cytotoxicity

For the determination of the in vitro cytotoxic effects of the triazenes in the presence of metabolic activation, a postmitochondrial liver supernatant, obtained by centrifuging a liver homogenate at 10,000 × *g* for 30 mins, was used. The livers were obtained from Sprague-Dawley rats, which received 0.5 g/liter of phenobarbital in their drinking water for 4 days before being killed. The livers were homogenized in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.4), using a Potter-Elvehjem tissue grinder. Protein concentration, determined by the method of Lowry et al (17) with bovine serum albumin as the standard, was adjusted to 40 mg/ml. The incubation mixture consisted of 1 ml of the buffer containing the drug, 20 mg of protein from the liver postmitochondrial supernatant, 12 μmols of $MgCl_2$, 0.8 μmol of NADP, 16 μmols of glucose-6-phosphate, and 0.35 unit of glucose-6-phosphate dehydrogenase (grade II from Boehringer-Mannheim), which was added to 3 ml of Dulbecco culture medium containing 4×10^6 TLX5 lymphoma cells and supplemented with 4% (vol/vol) fetal calf serum. After 2 hrs of incubation at 37°C with gentle shaking, cell viability was bioassayed by injecting 0.1-ml aliquots of the incubation mixture into groups of five animals whose survival time was recorded. These experiments were also performed without metabolic activation, omitting the liver supernatant and cofactors.

In Vitro Oxidative *N*-Demethylation and Hydrolysis

The incubation mixture was the same as above with the exception that the 3 ml of Dulbecco culture medium containing the tumor cells was replaced with 0.1 M potassium phosphate buffer (pH 7.4) containing 100 μmols of semicarbazide. After incubation for 2 hrs at 37°C with gentle shaking, the reaction was stopped by the addition of 1 ml of 20% (wt/vol) $ZnSO_4$ and 1 ml of saturated $Ba(OH)_2$ solution. After centrifugation, the Nash colorimetric assay for formaldehyde was carried out on the supernatant (18); the demethylation capacity of the system was checked by using aminopyrine as the substrate and was linear for at least 2 hrs.

The kinetic law of decomposition of the triazenes used, studied at pH 7.0 and 37°C in 65 mM phosphate buffer, was pseudo first-order with respect to the triazene concentration in all cases; the procedure was that reported by Kolar and Preussmann (19).

In Vivo Experiments

Approximately 10^5 TLX5 lymphoma cells or 10^6 P388 leukemia cells were transplanted ip into groups of five to ten CBA/lac or BDF_1 hybrids, respectively. The treatment was performed ip as indicated, using drugs freshly dissolved in 0.1 N $NaHCO_3$ and administered in 0.1 ml/10 g of animal weight. The survival time of the animals was recorded, and the total number of peritoneal tumor cells was measured at the end of treatment of TLX5 lymphoma-bearing mice. This was done by means of a Coulter Counter model ZF, after careful collection of tumor cells by repeated washing of the peritoneal cavity with isotonic NaCl solution.

The viability of peritoneal tumor cells at the end of treatment with repeated administrations of *p*-DM-COOK in mice bearing TLX5 lymphoma was bioassayed by ip transplantation of 10^5 tumor cells from the treated donors into normal recipients. The presence of clonogenic tumor cells in the brain was also measured at the end of treatment by transplanting the whole brains sc into normal mice. In both cases the survival time of the normal untreated recipients was recorded, which provided an indirect indication of the viability or number of tumor cells present in the bioassayed sample (20).

RESULTS

In Vivo Antitumor Activity

The effects of the three isomers on the survival time of CBA/lac mice bearing TLX5 lymphoma or of BDF_1 hybrids bearing P388 leukemia are reported in table 1. Each compound was tested using either single or repeated doses, including maximum tolerated doses. Both tumors were unresponsive to the treatment with *o*-DM-COOK with all the doses and treatment schedules employed. The antitumor activity of *p*-DM-COOK, in terms of the number of dose levels causing significant increases of survival time of the treated animals, was pronounced for both tumors; the activity of *m*-DM-COONa, though evident, was less marked.

The number of peritoneal tumor cells present when the animals were killed was not significantly modified by *p*-DM-COOK. The viability of the same tumor cells, bioassayed by transplantation into normal mice, was slightly reduced at the two higher doses employed. On the other hand, the survival time of mice transplanted with whole brains of the treated animals was significantly increased with all the doses used. The percent of animals having no detectable clonogenic tumor cells in their brains increased from 0% to 100% with increasing doses (table 2).

In Vitro Cytotoxicity

The cytotoxic activity of the tested compounds for TLX5 lymphoma cells, after 2 hrs of incubation in vitro

TABLE 1.—Effects of tested compounds on survival time of mice bearing TLX5 lymphoma or P388 leukemia

Tumor line	Compound	Treatment schedule	Active doses (mg/kg/day)[*]	Survival time (% T/C)[†]
TLX5 lymphoma	*o*-DM-COOK	Daily on Days 3–7	Inactive	
		Day 1 only	Inactive	
	m-DM-COONa	Daily on Days 3–7	100	146
		Day 1 only	200	128
	p-DM-COOK	Daily on Days 3–7	200,100,50,25	171,170,145,126
		Day 1 only	300	133
P388 leukemia	*o*-DM-COOK	Daily on Days 1–5	Inactive	
		Day 1 only	Inactive	
	m-DM-COONa	Daily on Days 1–5	200	187
		Day 1 only	200	134
	p-DM-COOK	Daily on Days 1–5	200,100,50	157,140,129
		Day 1 only	400,200	166,143

[*] Toxic deaths do not occur at the indicated active doses, whereas they are observed at the next unreported higher dose. For *o*-DM-COOK, toxic doses are 25–50 mg/kg.

[†] % T/C is the % ratio of the average survival time for each treated group to that of relevant controls. Only values significantly different from controls by the Student-Newmann-Keuls test (25) ($P < 0.05$) are reported.

TABLE 2.—Effects of *p*-DM-COOK on survival time and No. and viability of tumor cells in mice bearing ip TLX5 lymphoma[*]

Daily dose (mg/kg)	Survival time (days), mean ± SE	No. (× 10^6) of peritoneal tumor cells when killed, mean ± SE	Survival time (days) of mice inoculated with—		Survival at 4 mos[†]
			10^5 peritoneal tumor cells	Whole brains	
0	10.2 ± 0.2	697 ± 68	10.1 ± 0.3	10.8 ± 0.3	0/14
12.5	10.3 ± 0.3	501 ± 92	10.8 ± 0.2	14.0 ± 0.6[‡]	0/5
25	16.7 ± 1.2[‡]	457 ± 86	11.6 ± 0.4	20.7 ± 4.7[‡]	2/5
50	18.0 ± 1.5[‡]	471 ± 104	12.1 ± 0.2	15.3 ± 0.5[‡]	5/12
100	14.7 ± 0.3[‡]	546 ± 64	13.0 ± 0.2[‡]	21.6 ± 2.1[‡]	8/13
200	12.3 ± 0.3	574 ± 77	14.2 ± 0.6[‡]	120	5/5

[*] Groups of 5–14 tumor-bearing mice were treated ip with *p*-DM-COOK on Days 3–7 after tumor transplantation; 24 hrs after the last drug administration the animals were killed and their peritoneal tumor cells and whole brains were transplanted into normal recipients whose survival time is reported.

[†] No. of mice surviving at 4 mos/total No. transplanted with whole brains.

[‡] Significantly different from values for controls by the Student-Newmann-Keuls test (25) ($P < 0.05$).

at 37°C, is reported in figure 1. All three isomers were devoid of any cytotoxic effect for tumor cells when the incubation was performed in the presence of 10,000 × *g* rat liver supernatant and cofactors. When the drugs were added to the tumor cell suspension in the absence of metabolic activation, only *o*-DM-COOK at the two higher concentrations caused a significant cytotoxicity as evidenced by prolonged survival of the tumor recipients.

In Vitro Oxidative *N*-Demethylation and Hydrolysis

The percent of demethylation of the tested compounds, expressed as mols of formaldehyde produced during the in vitro incubation over the molar starting amount of dimethyltriazene, is reported in table 3. A 10%–13% demethylation was observed for *o*-DM-COOK, depending on the concentration of the drug used. *p*-DM-COOK was only weakly demethylated, whereas *m*-DM-COONa was almost completely resistant to in vitro oxidative *N*-demethylation.

The kinetic constants of hydrolysis to diazonium cations for the tested compounds are also reported in table 3. *o*-DM-COOK rapidly hydrolyzed in aqueous solution at neutral pH and 37°C; 64.8% of the initial amount was converted to diazonium cations within 2 hrs. *m*-DM-COONa and *p*-DM-COOK were markedly more resistant to hydrolysis; their relevant values were 7.7% and 2.8%, respectively.

DISCUSSION

The antitumor activity of the isomers of the salts of (3,3-dimethyl-1-triazeno)benzoic acid observed after in vivo administration to mice bearing either of two tumor

TABLE 3.—In vitro demethylation and kinetic parameters of hydrolysis of the tested compounds

Compound	Starting concentration, μg/ml	% demethylation, mean ± SE*	$K \times 10^{-3}$ (min^{-1})	Half-life, mins
o-DM-COOK	400	12.6 ± 4.8	8.710	79.58
	800	9.8 ± 2.3		
m-DM-COONa	400	1.5 ± 0.6	0.668	1037.74
	800	0.9 ± 0.5		
p-DM-COOK	400	3.0 ± 1.3	0.240	2889.46
	800	2.0 ± 0.8		

*Values were obtained from 4 separate determinations.

lines ranged from the inactivity of *o*-DM-COOK to the pronounced activity of *p*-DM-COOK; the activity of *m*-DM-COONa fell in between. For *p*-DM-COOK, the number of peritoneal tumor cells found at the end of the treatment in mice bearing TLX5 lymphoma was not significantly reduced, and their viability was slightly decreased only at the highest and nonoptimal doses. Considering the growth kinetics of this tumor, these reductions cannot be held responsible for the increase in survival time caused by *p*-DM-COOK. Indeed, a purely cytotoxic agent, cyclophosphamide, given at doses that increased the survival time of the mice to an extent similar to that caused by *p*-DM-COOK, caused the absence of detectable peritoneal tumor cells (14).

The lack of significant cytotoxicity of *p*-DM-COOK was confirmed when TLX5 tumor cells were incubated in vitro with the drug, either in the presence or absence of metabolic activation. The absence of significant cytotoxic effects has also been observed in cell cultures of Lewis lung carcinoma treated with 1.2 mg/ml of *p*-DM-COOK for several hours (unpublished results). *m*-DM-COONa was similarly devoid of cytotoxicity in vitro for TLX5 tumor cells , whereas *o*-DM-COOK significantly reduced the viability of TLX5 tumor cells after incubation in vitro in the absence of metabolic activation. This finding, however, should be interpreted in view of the fact that this compound has a half-life for hydrolysis to diazonium cations that is much shorter than that of its analogs examined here. Appreciable amounts of cytotoxic diazonium cations (11) can thus be produced during incubation only from this substance. The measurements of the oxidative *N*-demethylation of the tested isomers should be regarded as indicative only, since a complex kinetics of demethylation, presumably due to inhibition of the enzymatic system by the drugs, has been observed for dimethyltriazenes (12,21,22).

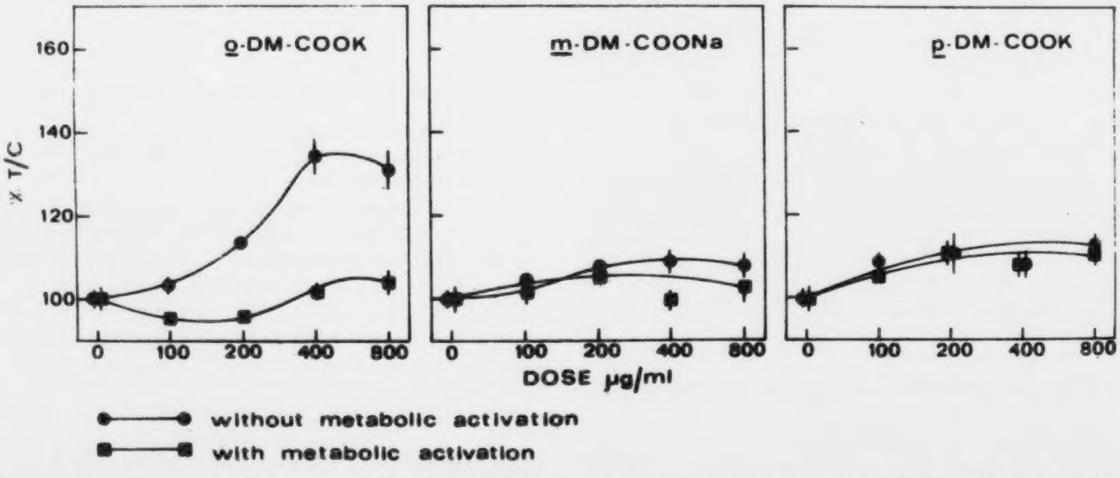

FIGURE 1.—In vitro cytotoxicity of the 3 isomeric derivatives of (3,3-dimethyl-1-triazeno)benzoic acid. Cytotoxic effects of the tested dimethyltriazenes result in increased survival time of the normal recipients inoculated with tumor cells treated in vitro, as compared with survival time of control animals receiving untreated tumor cells. Results obtained in 4 separate experiments have been averaged and are expressed as the % ratio (% T/C) of the survival time of mice receiving treated tumor cells over that for controls. Statistical analysis by Student-Newmann-Keuls test (25).

However, *o*-DM-COOK, though it is more subject to *N*-demethylation than are its isomers, was not cytotoxic in vitro in the presence of metabolic activation. These data indicate a lack of correlation between an increase in survival time of mice bearing tumors treated in vivo and the propensity of the isomers examined to generate diazonium cations or to undergo *N*-demethylation. It would thus appear that the in vivo effects of DM-COOK isomers are independent of the metabolic activation through the two pathways previously indicated and that these drugs might act directly on tumor cells.

As far as the mechanism involved in increasing the survival time of mice treated in vivo, a remarkable reduction of clonogenic tumor cells was observed in the brains of the treated animals. The ip administration of the drug did not significantly reduce the viability and number of peritoneal tumor cells, but the drug might prevent metastatic tumor cells from reaching the brain. Indeed, histologic examination of the brains of the treated animals revealed a virtual absence of leukemic infiltration, which was also evident in the liver and kidneys, as compared with untreated leukemic controls (to be published elsewhere). The results presented here are from representative experiments and were repeatedly obtained using the same tumor line as well as P388 leukemia. These findings and their relevance for the increased survival time of treated leukemic mice are currently under investigation in our laboratory. Furthermore, it is interesting to note that selective antimetastatic properties, consisting of the prevention of hematogenous dissemination of cells from solid tumors in mice, have already been observed for *p*-DM-COOK and related aryldimethyltriazenes and have been shown not to be caused by cytotoxic properties of the drugs (13,15,23,24).

The antimetastatic effects of *p*-DM-COOK for leukemic cells also, as suggested by the presently reported experiments, are of interest in view of the possibility of preventing the establishment of leukemic cells in the host's structures, such as those of the CNS, that are not easily accessible to chemotherapy with cytotoxic agents.

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BRIEF REPORTS

Phase II Evaluation of 4′-Epi-doxorubicin in Patients With Advanced Colorectal Carcinoma[1,2]

Richard Michaelson, Nancy Kemeny,* and Charles Young [3,4]

4′-Epi-doxorubicin (4′-epi-DX) is a new doxorubicin derivative that is more active than the parent compound against murine sarcoma virus tumors and Lewis lung carcinoma and may be less toxic. Thirty-five patients with advanced measurable colorectal carcinoma were treated with 4′-epi-DX (85 mg/m^2) every 3 weeks. The major toxic effect was leukopenia < 2000 cells/mm^3 in 28% of the patients. One of 29 patients (3%) had a partial response. At this dose and schedule, 4′-epi-DX has minimal activity in colorectal carcinoma. [Cancer Treat Rep 66:1757–1758, 1982]

4′-Epi-doxorubicin (4′-epi-DX) is a doxorubicin derivative differing from the parent compound by epimerization of the hydroxyl group at the 4′ position (1), which by lowering the affinity of the compound for DNA, results in higher intracellular levels of the drug compared to doxorubicin. 4′-Epi-DX was found to be as active as doxorubicin against Sarcoma 180, Gross' leukemia, L1210 and P388 ascitic leukemias, and 3H/He transplanted mammary carcinoma (1) and to be more active against sarcoma induced by murine sarcoma virus (MSV), metastatic Lewis lung carcinoma, and MS-2 sarcoma (2). Acute and chronic toxicity studies in normal and tumor-bearing mice and humans (2,3) have shown 4′-epi-DX to be less toxic than doxorubicin (2). Myelosuppression, vomiting, alopecia, and stomatitis were less severe than with comparable doses of doxorubicin (3). Two human studies (3,4) also demonstrated less cardiac toxicity, though it is too early to completely evaluate this. In one study, one of six patients with colorectal carcinoma responded. For these reasons, a phase II trial of 4′-epi-DX was initiated in patients with metastatic colorectal carcinoma.

MATERIALS AND METHODS

Thirty-five patients with measurable metastatic colorectal carcinoma were entered in the study. Patients were excluded from the study if they were > 85 years old; if they had a Karnofsky performance status < 50, wbc count < 4000/mm^3, platelet count $< 100{,}000$/mm^3, or bilirubin > 2.0 mg/liter; if there was evidence of congestive heart failure; or if they had cardiac ischemia or arrhythmia. Informed consent was obtained from all patients. Patients received a continuous iv infusion of 4′-epi-DX over 20 minutes every 3 weeks. The initial dose of 85 mg/m^2 was increased by 15 mg/m^2 if there was no hematologic toxicity and decreased by 15 mg/m^2 if wbc and platelet count nadirs were < 2000 or $< 75{,}000$/mm^3, respectively. A partial response was defined as a $> 50\%$ decrease in the sum of the products of two perpendicular diameters of all measurable disease. A minor response was defined as a $> 25\%$ decrease in all measurable disease. Stabilization was defined as a $< 25\%$ change, while disease progression was defined as a $> 25\%$ increase in measurable disease. An adequate trial was at least two doses of chemotherapy with toxicity or disease progression.

The median age of the patients was 56 years (range, 31–80), and the median Karnofsky performance status was 90 (range, 50–100). The median time from diagnosis of metastatic disease to entry in the study was 6 months (range, 1–46). Sites of measurable metastatic disease included liver (74%), lung (31%), abdomen (14%), and lymph node (6%). Twelve patients were previously untreated with chemotherapy or radiation therapy. Of the 23 previously treated patients, 21% had also received

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[1] Received Aug 3, 1981; revised May 11, 1982; accepted May 19, 1982.

[2] Supported in part by Public Health Service grant CA-05826 and contract N01-CM-97274 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Farmitalia.

[3] Solid Tumor Service (*R. Michaelson* and *N. Kemeny*) and Developmental Chemotherapy Service (*C. Young*), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

[4] We thank Jane Howard for assistance in record keeping and Pearl Mulraine for secretarial assistance.

**Reprint requests to:* Nancy Kemeny, MD, Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

prior radiotherapy. Prior chemotherapy consisted of 5-FU in 100% of the patients, a nitrosourea in 83%, and phase II agents in 21%. The mean duration of prior therapy was 11 months (range, 2–44).

RESULTS

Twenty-nine of the 35 patients who entered the study received an adequate trial. Of the six inadequately treated patients, four demonstrated marked disease progression before the second dose was administered, one refused a second dose after experiencing marked drug-related thrombocytopenia, and one who had been free of angina for 4 years noted the onset of angina-like chest pain for 1 hour several days after his first dose of the drug, without ECG changes.

The major hematologic toxic effect was leukopenia. Wbc counts $< 3000/\text{mm}^3$ were seen in 68% of the patients, and wbc counts $< 2000/\text{mm}^3$ were seen in 28%. The wbc count nadir was generally observed 10–14 days after administration of the drug, and full recovery occurred by Day 21 in all but two patients. Two patients who were neutropenic required hospitalization after fever developed. Thrombocytopenia was uncommon; 15% of the patients had a platelet count nadir $< 100{,}000/\text{mm}^3$, and 8% had a nadir $< 50{,}000/\text{mm}^3$.

Alopecia was common, with 85% of the patients having $> 50\%$ loss of hair. Nausea and vomiting, which occurred in 46% of the patients, was generally mild and lasted several hours. Moderate fatigue, lasting 3–21 days, was seen in 16 (60%) of the patients. Eight of the 16 patients noted a decrease in fatigue after the drug was discontinued. In four of the patients, symptomatic improvement correlated with response to treatment with other chemotherapy agents. Six (17%) of the patients noted rigors and fever several hours after drug administration. Symptoms of congestive heart failure were not observed, although only one patient received > 250 mg/m^2 of 4′-epi-DX, and this patient had 595 mg/m^2 without cardiac toxicity. None of the patients had mucositis, hepatic dysfunction, or dermatitis.

One of the 29 evaluable patients had a partial response to treatment. This patient had previously been treated with intrahepatic infusion of dactinomycin, 5-FU, and methotrexate. She was treated with 4′-epi-DX following intra-abdominal recurrence and responded with a 75% shrinkage in the size of an abdominal mass. The response lasted 5 months. One patient had disease stabilization by objective criteria but refused a third dose because of marked fatigue and a significant decline in performance status. All other patients had progressive disease.

DISCUSSION

In the treatment of metastatic colorectal carcinoma, doxorubicin as a single agent has been reported to have a partial response rate of 12% (six of 48 patients) in previously untreated patients and of 3% in previously treated patients (5). The true response rate to 4′-epi-DX in a group of previously treated and untreated patients was 3% (one of 29 patients), with a 95% confidence interval of 0%–17%. The one response was in a previously treated patient. It appears that 4′-epi-DX administered in this dose and schedule has a limited role, similar to that of doxorubicin, in the treatment of advanced colorectal carcinoma.

Recently, experiments on human colon tumors transplanted into mice demonstrated no activity with doxorubicin or 4′-epi-DX but definite activity with 4′-deoxydoxorubicin (6). Our observations are consistent with these studies and we will be initiating a study with 4′-deoxydoxorubicin.

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Phase I Study of 13-*cis*-Retinoic Acid Toxicity[1,2,3]

P. R. Band,[4,5,*] J. G. Besner,[6] R. Leclaire,[6] C. Girard,[5] G. Diorio,[4] M. Deschamps,[5] M. Gélinas,[5] and D. Larochelle[5]

A phase I study of 13-*cis*-retinoic acid was done in 16 patients with head and neck malignancies using a modified Fibonacci search scheme, with individual doses ranging from 20 to 120 mg/m². Drug doses > 60 mg/m² induced intense headaches, urethritis, desquamative dermatitis, vertigo, and ataxia. The severity of these side effects precludes the use of 13-*cis*-retinoic acid as a potential chemopreventive agent at doses > 60 mg/m². [Cancer Treat Rep 66:1759–1761, 1982]

Retinoids (vitamin A and its synthetic analogs) are essential for the normal growth and differentiation of epithelial tissues and prevent the development of a variety of epithelial tumors in experimental animals (1,2). In man, natural retinoids induce regressions of urinary bladder papillomas, oral leukoplasia, and fibrocystic masses of the breast (2,3). The structural synthetic analog of all-*trans* retinoic acid, 13-*cis*-retinoic acid (13-*cis*-RA), is less toxic than the parent compound (4), is effective in the prevention of lung, bladder, and colon carcinogenesis in rodents (5-7), and produces regressions of basal cell carcinoma in man (8). The clinical phase I study of 13-*cis*-RA described in this report was undertaken to establish the maximal tolerated dose of this drug for its potential use in a chemopreventive setting.

PATIENTS AND METHODS

Sixteen patients (15 males and one female; 33–78 years old) with stage I or II head and neck malignancies were admitted to the study after having fully recovered from the effects of surgery and/or radiation therapy, provided they had normal hemogram, creatinine clearance, and liver function tests. All patients gave informed consent. Baseline studies, repeated at 2-week intervals, included complete medical history and physical examination, complete hemogram, blood chemistries (SMA-12), serum electrolyte levels, and 24-hour creatinine clearance determination. Drug dose was based on body surface area and was given orally as a single daily dose for 3 consecutive months according to the modified Fibonacci search scheme shown in table 1. Each patient remained at his initially assigned dose level until removed from the study. Toxic effects were graded according to the scale shown in table 1.

RESULTS

Treatment characteristics are indicated in table 1. Two patients were treated with 13-*cis*-RA at a dose level of 20 mg/m², six at 35 mg/m², six at 60 mg/m², and one at 90 mg/m². One patient scheduled to receive 60 mg/m² of 13-*cis*-RA inadvertently took twice this amount as his first and only dose. Twelve patients received 13-*cis*-RA for 3 months without dose modification or interruption. Due to severe dryness and painful fissures of the lips, the dose was reduced from 60 to 35 mg/m² in one patient (No. 10). One patient (No. 2) refused treatment after 41 days in the study. Therapy was discontinued in one patient (No. 4) due to the occurrence of metastases, and in three when grade 3 toxic effects supervened. Grade 1 toxic effects occurred in all patients and, except for the severe toxic effects seen in patient 7, represented the only side effects observed at 13-*cis*-RA doses < 60 mg/m². One half of the patients treated with 13-*cis*-RA at doses of 60 mg/m² developed grade 2 toxic effects, whereas in the two patients receiving doses > 60 mg/m², severe toxic effects were encountered.

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[1] Received Oct 1, 1981; revised May 10, 1982; accepted May 18, 1982.

[2] A grant-in-aid was supplied by Hoffmann-La Roche Ltd, Canada.

[3] 13-*cis*-Retinoic acid was supplied by Hoffmann-La Roche, Nutley, NJ.

[4] Institut du Cancer de Montréal, Quebec, Canada.

[5] Hôpital Notre-Dame, Montreal, Quebec, Canada.

[6] Faculty of Pharmacy, University of Montreal, Quebec, Canada.

**Reprint requests to:* Pierre R. Band, MD, Cancer Control Agency of British Columbia, #700, 686 W Broadway, Vancouver, British Columbia, Canada V5Z 1G1.

TABLE 1.—Characteristics of 13-*cis*-RA treatment and toxic effects

Patient No./sex/ age (yrs)	Treatment duration (days)	13-*cis*-RA dose (mg)				Toxic effect	
		Per m^2	Daily	Cumulative to grade 1 toxicity	Total	Grade*	Type
1/M/76	92	20	30	1710	2760	1	Cheilitis
2/M/58	41	20	40	1040	1640	1	Cheilitis and conjunctivitis
3/M/65	92	35	50	1100	4600	1	Cheilitis, fissured lips, and xerosis
4/F/55	57	35	50	1450	2850	1	Cheilitis and xerosis
5/M/66	92	35	60	900	5520	1	Cheilitis, xerosis, and conjunctivitis
6/M/63	92	35	60	1320	5520	1	Cheilitis and xerosis
7/M/33	92	35	70	660	6440	3	Cheilitis, fissured lips, xerosis, blepharoconjunctivitis, urethritis, and exfoliative dermatitis
8/M/55	92	35	70	1540	6440	1	Cheilitis
9/M/65	92	60	100	800	9200	2	Cheilitis, xerosis, and skin rash
10/M/53	92	60	110	960	5960	1	Cheilitis, fissured lips, and xerosis
11/M/66	92	60	110	1650	10,120	2	Cheilitis, fissured lips, xerosis, conjunctivitis, and skin rash
12/M/37	92	60	120	960	11,040	1	Cheilitis, fissured lips, xerosis, and conjunctivitis
13/M/67	92	60	120	960	11,040	1	Cheilitis, fissured lips, xerosis, and conjunctivitis
14/M/64	92	60	130	1040	11,960	2	Cheilitis, xerosis, conjunctivitis, and increased serum alkaline phosphatase
15/M/49	15	90	160	1280	2400	3	Cheilitis, xerosis, conjunctivitis, fissured lips, skin rash, urethritis, headaches, and desquamative dermatitis
16/M/56	1	120	200	200	200	3	Same as No. 15, vertigo, and ataxia

*Toxicity grade: *grade 1*—cheilitis, xerosis, fissured lips, conjunctivitis, blepharoconjunctivitis; *grade 2*—skin rash, serum alkaline phosphatase increased > 50%; and *grade 3*—headaches, urethritis, and exfoliative dermatitis.

DISCUSSION

The side effects induced by 13-*cis*-RA observed in this study were mostly confined to the skin and mucous membranes. Xerosis and cheilitis were generally mild or moderate in nature and well-controlled with lubrication and topical dermatologic ointments. Skin rashes consisted of maculopapular, sometimes pruritic, eruptions localized over the trunk and limbs; this rash cleared spontaneously despite continuation of treatment at unmodified dose in two patients (Nos. 9 and 11). A mild transient increase in serum alkaline phosphatase was the only laboratory abnormality seen. Except for patient 16, grade 1 toxic effects occurred at an average cumulative dose of 1150 mg (range, 800–1700). Such a dose relationship was not apparent for grade 2 or 3 toxicity. All side effects rapidly disappeared at the cessation of treatment and no chronic toxicity was observed.

The retinoid 13-*cis*-RA has been used mostly in the treatment of various dermatoses and in basal cell carcinoma at an average maximum daily dose of 2 mg/kg (range, 0.5–7.4), with side effects similar to those reported in this study (8–10). Higher doses have been administered to patients with severe keratinizing dermatoses (8,9) or metastatic malignancies (11); the nature of these diseases may require acceptance of the increased toxicity associated with high drug dose.

It must be pointed out, however, that in the context of chemoprevention healthy individuals would be treated and toxic effects should be minimized. The efficacy of 13-*cis*-RA in preventing the progression of preneoplastic lesions to malignancy remains to be clinically proven. In our opinion, the severity of the toxic effects induced by 13-*cis*-RA precludes its use as a potential chemopreventive agent at doses > 60 mg/m^2.

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BRIEF REPORTS

High-Dose Medroxyprogesterone Acetate in Advanced Male Breast Cancer[1]

F. Pannuti,* A. Martoni, L. Busutti, M. Gramellini, and E. Piana[2]

Six males with advanced breast cancer were treated with high doses of medroxyprogesterone acetate. In five patients, the treatment induced a partial remission, with a median duration of 7 months. The treatment was well-tolerated. High doses of medroxyprogesterone acetate are effective and well-tolerated therapy for advanced male breast cancer. [Cancer Treat Rep 66:1763–1765, 1982]

Medroxyprogesterone acetate (MAP) is a semisynthetic progestational agent which, employed at high doses im (500, 1500, and 2000 mg/day for 30 days) in the treatment of advanced female breast cancer, induced objective remissions of 43%–45%, with little systemic toxicity (1–3). Previously, MAP used at low doses (< 500 mg/day) stimulated little interest in advanced female breast cancer patients (4–6).

The positive results obtained with high doses prompted us to employ this treatment in male patients with advanced breast cancer as well.

MATERIALS AND METHODS

Six male patients with advanced breast cancer, whose primary clinical characteristics are summarized in table 1, were treated with high doses of MAP. All of the patients had progressive disease. None had undergone antitumor therapy for at least 1 month. None had received hormonal therapy, and one patient had refused orchiectomy. All of the patients had a life expectancy of at least 2 months. Hormone receptor analysis was available only for patient 6, who had an estrogen receptor-positive primary tumor.

Treatment with high doses of MAP consisted of daily administration of the doses reported in table 1 for at least 25–30 days. Four patients received maintenance therapy: patients 1 and 4 were given 1500 and 500 mg im once or twice a week, respectively; patients 5 and 6 were given 1000 mg/day orally. Maintenance therapy was administered to all four patients until tolerance or disease progression.

Objective response was evaluated according to International Union Against Cancer criteria (7). Complete remission (CR) was defined as the disappearance of every tumor lesion for at least 6 months, and partial remission (PR) was defined as a ≥ 50% decrease in the surface area of all measurable tumor lesions or the partial healing of lytic bone lesions for at least 1 month.

RESULTS

As reported in table 1, five of the six patients had objective remission (CR + PR), for a median duration of 7 months. Patient 4 had a CR of pulmonary metastases and of both metastatic supraclavicular and laterocervical lymph nodes. The sixth patient, with liver metastases, had disease progression.

With regard to subjective remission, the three patients with osseous metastases (including the patient showing disease progression) had rapid and significant reduction of pain. The clinical side effects were a gluteal abscess (patient 1), moon face (patients 4 and 6), temporary impotence (patient 4), and erythematous reaction in the truncus which disappeared after the termination of treatment (patient 5).

DISCUSSION

Male breast cancer, like that in females, is considered to be hormone-sensitive. In the advanced stage of the disease, orchiectomy, which has been considered traditionally the first-choice treatment, induced objective remissions in an average of 60% of the patients (range, 45%–87%), with a mean duration of 20 months (8). Recently, using stricter criteria of response evaluation, this treatment induced objective remission in ten of 23 patients (43%) for 17 months (9). Of all the additive

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[1] Received Jan 13, 1982; revised Apr 29, 1982; accepted May 25, 1982.

[2] Divisions of Oncology (*F. Pannuti*, *A. Martoni*, and *E. Piana*) and Radiotherapy (*L. Busutti* and *M. Gramellini*), S. Orsola-M. Malpighi Hospital, Bologna, Italy.

**Reprint requests to:* Dr. Franco Pannuti, Division of Oncology, S. Orsola-M. Malpighi Hospital, Via Albertoni 15, 40138 Bologna, Italy.

Table 1.—High doses of MAP in advanced male breast cancer

Patient No.	Age (yrs)	Time from mastectomy (mos)	Disease-free interval (mos)	Previous treatment for metastases	Site of lesion	MAP					
						1st course					
						Dose/day (mg)	Total (mg)	Maintenance therapy	Objective response	Duration of remission (mos)	Survival (mos)
1	42	60	36	Polychemotherapy	Osseous	1500 im	40	Yes	PR	15	22
2	56	10	3	Polychemotherapy	Liver, lung, osseous	1500 im	45	—	Disease progression	—	2
3	63	6	5	—	Osseous, skin	1500 im	38	—	PR	24	27 +
4	42	84	48	—	Lung, lymph node	500 im	18	Yes	CR	7	13
5	63	—	—	—	$T_2N_3M_0$	2000 orally	60	Yes	PR	3 +	3 +
6	48	24	24	—	Lung	2000 orally	60	Yes	PR	6	24 +

hormonal therapies, diethylstilbestrol showed objective remission in 21 of 55 patients (10). Sporadic patients were responsive to calusterone therapy after the failure of orchiectomy (11) or 17-hydroxyprogesterone caproate treatment (12). Various investigators recently reported a few cases of advanced male breast cancer responsive to tamoxifen (13–17).

In the past, low doses of MAP were occasionally used in advanced cases of male breast cancer. Even though administration of MAP given at a dose of 100 mg three times a week im failed in two patients (6), oral administration (40–80 mg/day) induced objective remission of the disease in one of five patients when used as a single agent and in two of two when combined with diethylstilbestrol (5). In this study, objective remission was obtained in five of six patients. The remaining patient who had disease progression had diffuse liver metastases, which are notoriously unresponsive to hormonal treatment. The mechanism of antitumor activity of MAP is still unknown. Possibly, MAP acts directly on cancer cells interfering with hormonal receptors (18), whereby the inhibitory action of MAP on the hypothalamo-pituitary-gonadal axis (19) and hypothalamo-pituitary-adrenal-axis (20) would play a primary role. This process would involve a reduction in synthesis of human testicular and adrenal steroids.

The reported results show that high doses of MAP are effective in the treatment of advanced male breast cancer.

If our results are confirmed with more patients, high doses of MAP could be a possible alternative to estrogens and orchiectomy.

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BRIEF REPORTS

Persistent Hypomagnesemia Following Cisplatin Chemotherapy for Testicular Cancer[1]

Richard L. Schilsky,* Audrey Barlock, and Robert F. Ozols[2]

Twenty-nine patients with nonseminomatous germ cell tumors of the testis have been followed for a median of 1.9 years (range, 0.3–3.6) following completion of cisplatin-containing combination chemotherapy. Twenty-two of the 29 patients (76%) became hypomagnesemic during chemotherapy and, of these, 11 (50%) have remained persistently hypomagnesemic for as long as 3 years following discontinuation of cisplatin treatment. In the remaining patients, serum Mg levels returned to normal a median of 0.6 years (range, 0.2–2.1) after completion of chemotherapy. In eight of nine persistently hypomagnesemic patients studied, urinary Mg excretion was inappropriately elevated. Symptoms attributable to hypomagnesemia were rarely reported, although muscle cramps, twitching, or mild tremor were noted by the most severely hypomagnesemic patients. Continued followup of these patients is required to determine the effects of chronic hypomagnesemia in man. [Cancer Treat Rep 66:1767–1769, 1982]

Cisplatin is an inorganic platinum compound that has been of great value in the treatment of disseminated testicular cancer and that shows considerable promise in the treatment of ovarian cancer, carcinoma of the head and neck, and other tumors. Among the toxic effects associated with cisplatin chemotherapy, hypomagnesemia has emerged as a particularly common problem. The great majority of adults and virtually all children receiving cisplatin develop hypomagnesemia at some time during their treatment (1,2). This disorder may be accompanied by hypokalemia and hypocalcemia and may vary in severity from no symptoms to frank tetany. Hypomagnesemia appears to result from a cisplatin-induced defect in renal tubular Mg conservation with subsequent renal Mg wasting (1).

Though the occurrence of cisplatin-induced hypomagnesemia is now frequently recognized, considerable uncertainty exists concerning its natural history and proper management. Since our initial report of cisplatin-induced renal Mg wasting (1), we have had the opportunity to follow a number of patients with nonseminomatous germ cell tumors of the testis who were treated with a cisplatin-containing regimen. This report describes the occurrence and prolonged persistence of hypomagnesemia in such a patient population.

PATIENTS AND METHODS

From 1976 to 1980, patients with advanced testicular cancer referred to the Medicine Branch of the National Cancer Institute (Bethesda, MD) were treated with cisplatin-based combination chemotherapy. The patient characteristics and treatment regimens have been detailed previously (3). Twenty-nine patients have now been without chemotherapy and in complete remission for a median of 1.9 years (range, 0.3–3.6). At the completion of chemotherapy and at each subsequent clinic visit, blood was obtained for measurement of serum electrolytes, calcium, Mg, phosphate, BUN, creatinine, glucose, albumen, and uric acid. Periodically, a 24-hour urine collection was obtained and analyzed for volume, creatinine, and Mg concentrations.

RESULTS

Twenty-two of the 29 patients (76%) became hypomagnesemic (serum Mg < 1.4 mEq/liter) during chemotherapy and have remained persistently hypomagnesemic for a median of 1.3 years following discontinuation of cisplatin. The clinical characteristics of these patients are shown in table 1.

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[1] Received Mar 1,1982; revised May 20, 1982; accepted June 1, 1982.

[2] Medicine Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Richard L. Schilsky, MD, University of Missouri Health Sciences Center, Division of Hematology and Medical Oncology, 807 Stadium Rd, Rm N408, Columbia, MO 65212.

TABLE 1.—Persistent hypomagnesemia following cisplatin chemotherapy in 22 patients

	Median	Range
Total cisplatin administered (mg)	734	450–1100
Time following cisplatin therapy (yrs)	1.9	0.3–3.6
Total duration of hypomagnesemia (yrs)	2	0.3–4
Duration of hypomagnesemia after cisplatin therapy (yrs)	1.3	0.2–3.6
Mg nadir after cisplatin therapy (mEq/liter)	1.08	0.71–1.35
Current serum Mg (mEq/liter)	1.36	0.71–1.80

Though some patients have remained hypomagnesemic for as long as 3.6 years following discontinuation of cisplatin, serum Mg levels in 11 patients have thus far returned to normal. For these patients, serum Mg returned to normal levels at a median of 0.6 years after discontinuation of cisplatin treatment, although hypomagnesemia had persisted in some of the patients for as long as 2.1 years prior to resolution. In patients who presently remain hypomagnesemic, serum Mg levels have now been chronically low for a median of 1.8 years. This prolonged persistence of hypomagnesemia is not clearly related to either the total cisplatin dose administered or the Mg nadir during therapy.

In eight of nine persistently hypomagnesemic patients studied, urinary Mg excretion was inappropriately elevated, with a median urinary Mg level of 6.0 mEq/liter (range, 2.1–10.5). The remaining patient was able to conserve Mg normally (urinary Mg < 0.5 mEq/liter) in the presence of mild hypomagnesemia (serum Mg $= 1.20$ mEq/liter). Azotemia and hypocalcemia did not occur during the followup period. Median creatinine clearance for the entire group of patients was 83 ml/minute (range, 37–148), and serum calcium levels were normal in all patients. Similarly, symptoms attributable to hypomagnesemia were reported by only two patients. Both patients complained of muscle cramps, twitching, or mild tremor when serum Mg levels decreased to < 1 mEq/liter; however, frank tetany or other central nervous system symptoms did not occur.

DISCUSSION

With the introduction of effective combination chemotherapy, many young men with testicular cancer now achieve sustained disease-free remission and probable cure. As these individuals look forward to the resumption of a normal life-style, the chronic and potential late toxic effects of treatment become increasingly important. The nephrotoxicity associated with cisplatin chemotherapy is manifest not only as a decrease in glomerular filtration rate but also, in many patients, as a specific defect in renal tubular Mg conservation (4,5).

This study clearly demonstrates that in many patients, hypomagnesemia and renal Mg wasting are problems that may persist for years following completion of cisplatin chemotherapy. Though rarely symptomatic in the absence of acute illness, patients may be at risk for the development of tetany during any intervening illness that limits oral intake or promotes excessive gastrointestinal losses of Mg. Further, the effects of chronic hypomagnesemia in man are unknown. Mg is a vital intracellular cation, important for a number of biologic processes. Apart from the effects of acute hypomagnesemia on neuromuscular irritability, Mg deficiency has been implicated as a cause of cardiac arrhythmias, esophageal spasm, and hemolytic anemia (6). Clearly, continued followup of these patients is necessary to assess the potential late effects of chronic hypomagnesemia.

The value of oral Mg supplementation for asymptomatic patients has not been established. Since the primary defect is renal wasting of Mg, it is not clear that Mg supplementation can effectively raise the low serum levels. Of the 22 persistently hypomagnesemic patients in the present study, 13 received continuous oral Mg supplements without a discernible effect on serum Mg levels. Further, of the 11 patients who have recovered from hypomagnesemia, five had received Mg supplements, yet the median duration of hypomagnesemia prior to recovery for these patients (2 years) was, in fact, longer than for the group who returned to normal but never received supplemental Mg (1.5 years). Thus, it would appear that chronic Mg supplementation is not effective in raising serum Mg levels or in hastening the resolution of hypomagnesemia. Whether continuous oral Mg therapy plays any role in preventing the development of symptomatic hypomagnesemia is uncertain.

Symptomatic patients should initially be treated with parenteral $MgSO_4$. These individuals may then require a dietary Mg supplement in an attempt to prevent recurrence of symptoms. A 24-hour urine collection should first be obtained for determination of creatinine clearance and Mg content. The dose of oral Mg prescribed should then be determined based on the extent of urinary losses and after consideration of the fact that the average daily diet contains approximately 25 mEq of Mg, of which only 30%–50% is absorbed (7). It is rarely necessary to raise the serum Mg level to normal since symptoms are uncommon at serum levels > 1.0 mEq/liter.

Cisplatin-induced hypomagnesemia must now be regarded as a chronic and potentially disabling toxic effect in many patients who have been cured of testicular cancer. The long-term effects of hypomagnesemia in this patient population remain to be established. Unfortunately, there are no known ways to prevent cisplatin-induced hypomagnesemia. It will be important, therefore, to evaluate all forthcoming cisplatin analogs in the hope of developing an effective new agent which is nontoxic to the renal tubule.

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CLINICAL TRIALS SUMMARIES

Tamoxifen in the Treatment of Metastatic Malignant Melanoma[1]

John Wagstaff, Nick Thatcher,* Elaine Rankin, and Derek Crowther[2]

Hormonal influences are thought to be of clinical importance in malignant melanoma (1), and cytoplasmic estrogen receptors in malignant melanoma have been described (2). These considerations led to the use of tamoxifen in the treatment of malignant melanoma, and response rates of 15%–37% have been reported, with response occurring mainly in cutaneous and subcutaneous lesions (2,3). Tamoxifen, a relatively nontoxic, convenient oral agent was prescribed to patients with widely metastatic melanoma who had failed with dactinomycin and dacarbazine chemotherapy.

PATIENTS AND METHODS

Twenty-three consecutive patients who had stage III melanoma (Karnofsky score ⩾ 50) with progressing tumor despite chemotherapy with a 5-day regimen of dacarbazine and dactinomycin were entered in the study (table 1). Tamoxifen (10 mg twice daily) was administered until further disease progression occurred. Standard response criteria were used.

RESULTS

Toxicity from tamoxifen was minimal: the drug was not stopped in any patient because of side effects. No patient satisfied the criteria for response: one patient has stable disease and continues to receive tamoxifen at Day 60.

DISCUSSION

The study failed to confirm the efficacy of tamoxifen in malignant melanoma that had been previously reported (2,3). However, the treated patients were a poor-risk group with widespread metastases (both visceral and nonvisceral) and had been previously treated with chemotherapy. Moreover, nine patients received the agent for < 4 weeks. Tamoxifen may have a role in melanoma but not in the poor-risk patients with advanced disease comprising the present study group.

TABLE 1.—Patient characteristics

Characteristic	
No. of males/females	9/14*
Mean age in yrs (range): 53 (29–72)	
No. of patients with systemic metastasis	23†
Duration of treatment (days)	
Median	30
Range	6–118
Median survival from start of treatment (days)	44‡

*5 patients were premenopausal.

†Metastatic pattern: liver, 8; lung, 13; brain, 2; nodal, 18; bone, 11; and cutaneous, 19.

‡5 patients were alive at 2, 3, 6, 9, and 9 mos.

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[2] Cancer Research Campaign, Department of Medical Oncology, University of Manchester and Christie Hospital and Holt Radium Institute, England.

**Reprint requests to:* Dr. Nick Thatcher, Cancer Research Campaign, Department of Medical Oncology, University of Manchester and Christie Hospital and Holt Radium Institute, Wilmslow Rd, Manchester M20 9BX, England.

Phase II Study of Medroxyprogesterone Acetate With Tamoxifen in Advanced Renal Cell Cancer[1,2]

Joachim Z. Fuks,* Joseph Aisner, David A. Van Echo, and Peter H. Wiernik[3]

A small but finite degree of antitumor activity for antiestrogen against renal cell carcinoma has been reported (1–3). Although both progesterone and tamoxifen exhibit antiestrogen activity, these agents may have different mechanisms of effect (4,5), and thus the two hormonal agents could interact to produce greater (or lesser) antitumor response.

Fourteen patients with measurable histologically confirmed renal cell carcinoma not amenable to curative surgery and with Eastern Cooperative Oncology Group performance status ≤ 3 who had not received prior hormonal therapy were entered in the trial.

Patients received tamoxifen at a dose of 10 mg orally 3 times a day and medroxyprogesterone acetate at a dose of 1.0 g/week im for at least 4 weeks. If objective remission or disease stabilization was achieved, therapy was continued until disease progression occurred. Tumor progression was defined as an increase of at least 50% in the size of any measurable lesions or the appearance of new lesions; when tumor progression occurred, therapy was stopped. Pretreatment patient characteristics are outlined in table 1.

TABLE 1.—Pretreatment patient characteristics

Characteristic	No. of patients
Entered/evaluated	14/14
Median age in yrs (range): 57 (25–72)	
Males/females	8/4
Previous treatment	
Nephrectomy alone	6
Chemotherapy alone	1
Nephrectomy and chemotherapy	4
None	3
Metastatic site	
Lung	9
Bone	8
Liver	5

All patients were evaluable for response and toxicity. A total of 121 doses of medroxyprogesterone acetate and 2508 doses of tamoxifen were given to the 14 patients, with a median of six (range, four to 28) and 154 (range, 112–588) doses of medroxyprogesterone acetate and tamoxifen, respectively, per patient. There were no objective responses. Calculated from the first day of treatment, four patients had initial disease stabilization for 4 weeks followed by disease progression, eight patients had disease stabilization for 6–10 weeks, and an additional two patients had disease stabilization for 21 and 28 weeks followed by disease progression. Flushing (three patients), headaches (two), fluid retention (three), and nausea (one) were the only toxic effects observed during this trial. The toxicity was insidious, and its severity was not enhanced on days of medroxyprogesterone acetate administration. Ten patients experienced no side effects.

No antitumor response observed among these 14 patients excludes a 20% level of activity with 90% confidence limits. It is conceivable, however, that the presence of hormone receptors on tumor cells is necessary for hormonal antitumor response or that the reduction of estrogen receptor concentrations by the progestin may have blocked the effectiveness of the antiestrogen. Thus, further studies of hormonal therapy for advanced renal cell carcinoma correlated with estrogen and progesterone receptor concentration of tumor cells may be warranted.

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[1] Received Jan 8, 1982; revised May 21, 1982; accepted May 25, 1982.

[2] Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.

**Reprint requests to:* Joachim Z. Fuks, MD, University of Maryland Cancer Center, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

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CLINICAL TRIALS SUMMARIES

Phase II Clinical Study of Yoshi 864 in Epithelial Ovarian Carcinoma: A Gynecologic Oncology Group Study[1,2]

M. Slavik,[3] H. Muss,[4] J. A. Blessing,[5] and G. Delgado[6,*]

Yoshi 864 is a busulfan analog active against experimental tumors resistant to mechlorethamine (1). This drug was reported to have some activity in ovarian carcinoma in a broad phase II study at doses causing minimal toxic reactions (2). Therefore, a group-wide phase II study of Yoshi 864 in epithelial ovarian carcinoma, using the same schedule, was undertaken. Thirty-three patients with advanced, recurrent, histologically confirmed epithelial ovarian carcinoma not amenable to further surgery, radiotherapy, or higher priority chemotherapy were evaluated. All patients had measurable disease. Neither ascites nor pleural effusion alone was considered measurable for purposes of this study; sonography was accepted if the lesion was sharply defined during initial examination. All patients had a Gynecologic Oncology Group (GOG) performance status $\leqslant 3$ (median, 1). Patients had to have recovered from the nadir blood cell counts from previous therapy and had to have a granulocyte count $\geqslant 1500/\text{mm}^3$ and a platelet count $\geqslant 100{,}000/\text{mm}^3$ before study entry. A BUN < 25 mg/100 ml, serum creatinine < 1.5 mg/100 ml, bilirubin < 1.0 mg/100 ml, and SGOT < 50 IU/liter were required. Standard response criteria were used (3). Complete response was defined as the disappearance of all gross evidence of disease for at least 1 month, while partial response was a $\geqslant 50\%$ reduction in the sum of the products of the largest diameter and the perpendicular diameter of all measurable lesions. Progressive disease was defined as a $\geqslant 50\%$ increase in the sum of these products or the appearance of any new lesions within 3 months of study. Stable disease was defined as a response not meeting criteria for complete or partial remission or progressive disease.

Yoshi 864 was administered by slow iv push at a dose of 2.0 mg/kg/day for 5 days, repeated every 6 weeks. Dose adjustment to 1.5 mg/kg/day was required when significant bone marrow toxicity occurred in the previous course.

Patient characteristics, response to therapy, and toxic effects are summarized in table 1. No objective tumor regressions occurred. Of the 33 patients, six had stable disease, 27 had progressive disease, and 18 received only a single course of the drug because of increasing disease. The most serious toxic effect was thrombocytopenia, reported in 11 patients with a median nadir of 49,000 cells/mm^3 (range, 5000–140,000) and a median occurrence in course 1 (range, 1–2). Leukopenia, with a median nadir of 2700 cells/mm^3 (range, 1300–3900), was reported in 14 patients after median course 1 (range, 1–2). Neurotoxicity, which was manifested as lethargy, drowsiness, disorientation, malaise, or paresthesia, occurred in five patients. The other toxic effects included nausea and vomiting in ten patients and fever and alopecia in individual cases.

We conclude that Yoshi 864 at this dose schedule is not an active drug in the treatment of patients with advanced epithelial ovarian cancer recurring after previous treatment with chemotherapy.

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[1] Received Feb 1, 1982; revised May 13, 1982; accepted June 14, 1982.

[2] This study was conducted by the Gynecologic Oncology Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in this study: Georgetown University Hospital, Washington, DC (CA-16938); Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC (CA-21946); Indiana University Medical Center, Indianapolis (CA-21720); University of Minnesota Medical School, Minneapolis (CA-23088); University of Pittsburgh School of Medicine, PA; University of Texas Health Science Center at Dallas (CA-28160); Tufts New England Medical Center, Boston, MA; University of Rochester Medical Center, NY (CA-12482); The Milton S. Hershey School of Medicine of Pennsylvania State University, Hershey (CA-16386); Walter Reed Army Medical Center, Washington, DC (CA-23501); University of North Carolina School of Medicine, Chapel Hill (CA-23073); and the Illinois Cancer Council, Chicago (CA-27806).

[3] Cancer Research and Treatment Center, University of New Mexico, Albuquerque.

[4] Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

[5] Roswell Park Memorial Institute, Buffalo, NY.

[6] Georgetown University, Washington, DC.

[*] *Reprint requests to:* Gynecologic Oncology Group Headquarters, Suite 430, 1234 Market St, Philadelphia, PA 19107.

TABLE 1.—Phase II study of Yoshi 864 in advanced epithelial ovarian carcinoma

Age (yrs)	GOG performance status	Previous treatment*	Response to Yoshi 864†	No. of courses of Yoshi 864	Toxic effects
25	0	MELPH, ADR-CTX-CIS	ID	2	Leukopenia (2500 cells/mm^3)
38	1	XRT, ADR-CTX, Megace-tamoxifen	SD	2	Leukopenia (3100 cells/mm^3)
44	0	ADR-CTX	ID	1	Leukopenia (2300 cells/mm^3), lethargy, thrombocytopenia (49,000 cells/mm^3)
44	2	ADR-CTX	ID	1	Leukopenia (3944 cells/mm^3)
49	0	ADR-CTX	ID	2	—
50	0	MELPH, HMM-CTX-ADR-CIS	ID	2	Leukopenia (3900 cells/mm^3), nausea
50	0	MELPH, *C. parvum*, CIS-CTX	ID	1	Disorientation, thrombocytopenia (5000 cells/mm^3), nausea, vomiting
52	1	MELPH, ADR-CTX-CIS	ID	1	Nausea
52	2	ADR-CTX	ID	2	—
53	0	MELPH, P-32, CIS, CTX-HMM, MTX-5-FU	SD	2	Paresthesia, nausea, anemia, leukopenia (2000 cells/mm^3), thrombocytopenia (43,000 cells/mm^3)
53	0	XRT-MELPH, ADR-CTX, CIS	SD	3	Leukopenia (3300 cells/mm^3), thrombocytopenia (98,000 cells/mm^3)
53	2	ADR-CTX, XRT, CIS	ID	1	—
55	1	ADR-CTX-CIS	SD	4	Leukopenia (1300 cells/mm^3), thrombocytopenia (21,000 cells/mm^3), lethargy, fever (101°F)
55	3	ADR-CTX	ID	1	Lethargy, drowsiness
57	1	ADR-CTX-CIS	ID	1	—
58	0	MELPH, ADR-CTX-CIS	SD	3	Nausea
58	1	MELPH, ADR-CTX-CIS, HMM	ID	1	Nausea, vomiting
58	2	MELPH, XRT, CIS	ID	1	Leukopenia (3900 cells/mm^3), thrombocytopenia (35,000 cells/mm^3)
59	0	ADR-CTX, VLB-BLEO-CIS	ID	2	Alopecia, thrombocytopenia (140,000 cells/mm^3)
59	0	XRT, MELPH-HMM	ID	2	Leukopenia (2200 cells/mm^3), thrombocytopenia (57,000 cells/mm^3)
59	1	MELPH, ADR-HMM-CIS	ID	1	Severe anemia
61	0	MELPH, CIS	SD	2	Leukopenia (2300 cells/mm^3), thrombocytopenia (80,000 cells/mm^3)
62	0	XRT, ADR-CTX-CIS, HMM, MTX-5-FU	ID	2	Nausea, thrombocytopenia (10,000 cells/mm^3), hemiparesis
62	0	ADR-CTX	ID	1	—
62	1	ADR-CTX-CIS	ID	1	—
62	2	ADR-CTX-CIS	ID	1	—
63	1	MELPH, HMM-5-FU-CTX, CIS-ADR	ID	1	—
65	0	ADR-CTX-CIS	ID	1	Drowsiness, malaise, fever (101°F)
65	3	MELPH	ID	1	Leukopenia (2900 cells/mm^3)
66	0	XRT, CTX-MTX-5-FU, ADR-CIS, HMM	ID	2	Leukopenia (2700 cells/mm^3), thrombocytopenia (133,000 cells/mm^3)
66	3	XRT, MELPH-5-FU-Megace	ID	1	—
70	3	ADR-CTX-CIS, HMM	ID	1	Nausea
75	1	HMM-CTX-MTX-5-FU, ADR-CTX-CIS, XRT	ID	2	Leukopenia (2900 cells/mm^3)

*MELPH = melphalan; ADR = doxorubicin; CTX = cyclophosphamide; CIS = cisplatin; XRT = radiation therapy; HMM = hexamethylmelamine; *C. parvum* = *Corynebacterium parvum*; MTX = methotrexate; VLB = vinblastine; and BLEO = bleomycin.

†ID = increasing disease; SD = stable disease.

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Phase II Trial of Mitoxantrone in Previously Untreated Patients With Colorectal Adenocarcinoma: A Southwest Oncology Group Study[1,2]

John D. Cowan,[3,*] Daniel D. Von Hoff,[4] Bruce McDonald,[5] Robert W. Talley,[6] Joseph D. McCracken,[3] and Timothy Chen[7]

Mitoxantrone is a new bis anthraquinone that, in preclinical screening, has had activity in a number of animal tumor systems, including Colon 25 and Colon 33 tumors (1). A phase I study of mitoxantrone using a single dose every 3 weeks has shown that the dose-limiting toxicity of the drug is leukopenia (2). Based on these data, the present phase II study was initiated to determine the therapeutic value of mitoxantrone in patients with metastatic colon cancer who had not been previously exposed to cytotoxic chemotherapy.

PATIENTS AND METHODS

Patients with histologically confirmed metastatic adenocarcinoma of the colon, without prior exposure to cytotoxic chemotherapy, were entered in this trial. Eligibility criteria included informed consent, a measurable lesion, life expectancy of at least 10 weeks, performance status ≤ 3 according to the Southwest Oncology Group (SWOG) criteria, wbc count $\geq 4000/\mu l$, platelet count $\geq 100,000/\mu l$, serum creatinine ≤ 1.5 mg/dl, and bilirubin ≤ 3 mg/dl.

Patient characteristics are shown in table 1.

On the basis of a phase I study (2), mitoxantrone was administered iv at the initial dose of 12 mg/m^2 over 30 minutes in 150 ml of 5% dextrose in water. The dose was repeated every 3 weeks if the wbc count was $\geq 4000/\mu l$ and the platelet count was $\geq 100,000/\mu l$. An adequate trial was considered to be two courses. Dose modifications were encouraged and were based on the wbc and platelet count nadirs. If the wbc count nadir was $\geq 4000/\mu l$ and the platelet count nadir was $\geq 100,000/\mu l$, the dose was increased by 10%. If the wbc count nadir was $< 2000/\mu l$ and/or the platelet count nadir was $< 75,000/\mu l$, the dose was decreased by 17%.

Standard objective response criteria of SWOG were used.

RESULTS

Thirty-four of 36 patients entered in the study were eligible for toxicity evaluation, with 33 of these being evaluable for response (table 1). Two patients died due to progressive colon cancer during their first 21 days in the study, and one refused further therapy after one course of mitoxantrone. Toxicity data demonstrated that the major toxic effect of mitoxantrone was leukopenia (granulocytopenia). The one neutropenic episode associated with infection resolved without sequelae. Only one of the four patients who had received prior pelvic radiation therapy had a granulocyte count nadir $< 1000/\mu l$ ($900/\mu l$). Nonhematologic toxicity was mild. No cardiac, renal, or hepatic toxic effects were reported, and there were no drug-related deaths.

There were no complete responses, but there was one partial response in the 33 evaluable patients. The partial response occurred in a 71-year-old female with measurable disease limited to the lungs; it lasted > 36 weeks.

DISCUSSION

Our data confirmed that leukopenia is the major toxic effect of mitoxantrone, even in patients not previously exposed to cytotoxic chemotherapy. Although preclinical screening data (1) had suggested that mitoxantrone

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[1] Received Feb 17, 1982; revised May 12, 1982; accepted May 26, 1982.

[2] Supported in part by Public Health Service grants CA-22411, CA-22433, CA-04915, CA-12644, CA-14028, CA-03392, CA-03096, CA-27057, CA-28862, CA-13238, CA-04919, CA-16957, CA-12213, CA-20319, CA-26766, CA-03389, CA-12104, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Brooke Army Medical Center, San Antonio, TX.

[4] University of Texas Health Science Center at San Antonio.

[5] Ontario Cancer Foundation, Windsor, Canada.

[6] Henry Ford Hospital, Detroit, MI.

[7] Southwest Oncology Group Biostatistical Center, Houston, TX.

[8] We thank Ms. Robbie Fuqua for preparation of the manuscript.

**Reprint requests to:* Southwest Oncology Group (SWOG-7940/44), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

might have a clinically significant effect on colorectal adenocarcinoma, our phase II study identified no such effect. In a human tumor cloning system, mitoxantrone had an antineoplastic effect against a number of human tumor histologies (> 50% decrease in colony formation) but had no activity in the 12 evaluable colon cancers (3). This suggests that the soft-agar human tumor cloning assay of Hamburger and Salmon (4) warrants continued investigation as a screening tool for antitumor agents and as a predictor of response or lack of response in the clinic.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 60 (41–86)	
Sex	
Males	17
Females	19
Median performance status (range): 1 (0–3)	
Prior therapy	
Pelvic radiation	4
Chemotherapy or immunotherapy	0
Evaluable for—	
Toxicity	34
Response	33
No. of evaluable courses: 143	
1–3 courses	19
4–6 courses	9
7–13 courses	6
Dose modifications (mg/m^2)	
Increase to—	
13.2	11
14.5	1
20.0	1
Decrease to—	
10	1
8	1
Toxicity	
Hematologic[*]	
PML count nadir (cells/μl)	
500–999	12
< 500	2
Nonhematologic	
Nausea	9
Alopecia	1
Stomatitis	1
Disease response	
Partial	1
Stable[†]	15
Progressive	17

[*] Median wbc count nadir = 3000 cells/μl (range, 1400–6400); median polymorphonuclear leukocyte (PML) count nadir = 1174 cells/μl (range, 378–4736); and median platelet count nadir = 225,000 cells/μl (range, 107,000–500,000).

[†] Median duration = 16 wks (range, 12–39).

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CLINICAL TRIALS SUMMARIES

Yoshi 864 Plus Medroxyprogesterone Acetate in Adenocarcinoma of the Kidney: A Southwest Oncology Group Study[1,2]

Stanley J. Altman,[3] Ronald L. Stephens,[4] and John D. Bonnet[5,*]

Systemic chemotherapy has had little or no effect in palliating disseminated adenocarcinoma of the kidney.

In 21 patients treated with medroxyprogesterone acetate (MPA) parenterally, two responses were noted (1). Bloom (2), reporting primarily on MPA and androgen therapy (the latter for MPA failures), observed an overall objective response rate of 16% in 80 patients who had response lasting from 2 to 35 months; only one of the responses was to androgens.

Yoshi 864 was synthesized in an effort to develop an alkylating agent effective in tumors resistant to mechlorethamine (3). The Central Oncology Group (COG) established a dose schedule of 2 mg/kg/day × 5 by iv push, repeated every 7 weeks (4). In a phase II study (5), there were no objective responses among 11 evaluable patients treated for adenocarcinoma of the kidney. However, in a COG pilot study, three of four patients with adenocarcinoma of the kidney, treated with Yoshi 864 and MPA at a dose of 400 mg/week im, achieved partial responses lasting from 2 to 6 months. Because of the promising pilot data, the Southwest Oncology Group decided to further evaluate this combination.

PATIENTS AND METHODS

Thirty-one adult patients (19 males and 12 females) were studied. Twenty-nine were nephrectomized, and six had had prior chemotherapy or hormonal therapy. Twenty-six were patients considered to be good risk and five were considered to be poor risk, which was defined as having any of the following qualities: age > 65 years; radiotherapy in > 50% of the marrow; or bone marrow metastases. All patients had histologically proven diagnosis and objectively measurable disease. Additional requirements were: wbc count > 4000/mm^3 and platelet count > 100,000/mm^3; BUN < 30 mg/100 ml, creatinine < 1.5 mg/100 mg, and total bilirubin < 4 mg/100 ml; no prior MPA or Yoshi 864; recovery from toxicity of prior treatment; life expectancy of at least 90 days; and written informed consent.

Good-risk and poor-risk patients were given 80 and 60 mg/m^2, respectively, of Yoshi 864 by iv push daily for 5 days and 400 mg/week im of MPA. The course of Yoshi 864 was repeated every 49 days if the wbc count was ≥ 4000/mm^3, and the platelet count was ≥ 100,000/mm^3. Blood cell counts were performed on Days 1, 22, and 50. Blood chemistries were obtained on Day 1 and every 49 days if abnormal or at the termination of study if initially normal. A minimum adequate trial was 7 weeks of treatment and follow-up.

Tumor measurements were recorded in centimeters and consisted of the longest diameter and the widest perpendicular diameter. Liver measurements were made at the xyphoid and 8 cm on either side.

Partial remission was a ≥ 50% decrease in the sum of the products of all diameters of measured lesions and, for liver metastases, a 50% reduction in the sums of measurements below the costal margin, with no increase in any lesion or the appearance of new lesions.

RESULTS

Sufficient data were available for analysis of 30 patients. There were two partial responses (one male, one female) lasting for at least 4 and 16 weeks in patients with lung metastases whose only prior therapy was nephrectomy. Toxicity was minimal (table 1).

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[1] Received Feb 25, 1982; revised June 10, 1982; accepted June 16, 1982.

[2] Supported in part by Public Health Service grants CA-13238, CA-12644, CA-22411, CA-21116, CA-03096, CA-27057, CA-03392, CA-22433, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] LDS Hospital and University of Utah Medical Center, Salt Lake City.

[4] University of Kansas Medical Center, Kansas City.

[5] Scott and White Clinic, Temple, TX.

[*] *Reprint requests to:* The Southwest Oncology Group, Operations Office, Cancer Therapy and Research Center, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Toxic effects in 30 patients

Toxic effect	Grade	No. of patients
Leukopenia	1 (3000–3999 wbcs/mm^3)	8
	2 (2000–2999 wbcs/mm^3)	5
	3 (1000–1999 wbcs/mm^3)	2
Thrombocytopenia	1 (75,000–99,999 platelets/mm^3)	2
	2 (50,000–74,999 platelets/mm^3)	4
	3 (25,000–49,999 platelets/mm^3)	2
Anemia	1 (Hgb 9–9.9 g/100 ml)	5
	2 (Hgb 7–8.9 g/100 ml)	2
	3 (Hgb 5–6.9 g/100 ml)	2
Nausea	1 (minimal)	1
Diarrhea	1 (minimal)	1
Somnolence	1 (minimal)	1
Fluid retention	1 (minimal)	1
Mucositis	1 (erythema)	1
Decreased libido		1

DISCUSSION

There were brief partial responses in two patients (7%). The patient whose response lasted 4 weeks was removed from the study because of the appearance of symptomatic brain metastases, although his lung lesions were still responding. As used in this study, MPA plus Yoshi 864 is no better than MPA alone.

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CLINICAL TRIALS SUMMARIES

Failure of 5-Day Vinblastine Infusion in the Treatment of Patients With Advanced Refractory Breast Cancer[1]

Ian Tannock,[2,*] Charles Erlichman,[2] Danièle Perrault,[2] Ian Quirt,[2] and Michael King[3]

In a recent publication, Yap et al (1) reported a 40% response rate among 30 evaluable patients with advanced breast cancer who had been extensively treated with other drugs. Because of this experience we initiated similar treatment in 17 patients with advanced breast cancer. We observed no responses and considerable toxicity.

All of our patients had advanced symptomatic metastatic disease, and all had received extensive prior therapy (table 1). Nine of the patients had received vincristine, but only two had received prior vinblastine, given as bolus injections with mitomycin. All patients had measurable disease, and all had normal wbc and platelet counts on entry in the study. Abnormal hepatic function did not contraindicate treatment.

Patients received a continuous 5-day iv infusion of vinblastine at starting doses of 1.2–1.8 mg/m^2/day. Courses were given at 3-week intervals, and in the absence of toxicity, the dose was escalated by 0.2 mg/m^2/day in successive courses. All patients received one full 5-day course, and 11 patients received at least two courses (table 1). Treatment was discontinued because of disease progression in 15 of the patients and because of toxic effects in two others. Patients with toxic reactions had a reduction in duration of treatment to 4 or 3 days on second and subsequent courses. Blood cell counts were normal at the time of restarting treatment in all patients except one.

Patients were evaluated at 3-week intervals. No patient responded to treatment. There was, however, considerable myelotoxicity, with one drug-related death from infection during myelosuppression following the initial course of vinblastine. The polymorphonuclear cell count decreased to $< 1000/mm^3$ in nine patients; two of them had platelet counts $< 50,000/mm^3$. Toxicity was not clearly related to dose and usually occurred after the first course. One patient experienced a sudden onset of right leg weakness 6 days after the end of her second course of treatment which then gradually improved: this may have been due to drug toxicity but the patient was known to have CNS metastases that were in remission after cranial irradiation.

In our study vinblastine given by infusion to patients with advanced refractory metastatic breast cancer was ineffective and toxic. Our response rate was 0 (95%

TABLE 1.—Characteristics of 17 patients treated with vinblastine infusion

Characteristic	No. of patients
Sites of disease	
Bone	13
Soft tissue (skin, nodes, etc)	16
Viscera (liver, lung)	9
CNS*	2
Eastern Cooperative Oncology Group performance status	
1	3
2	11
3	3
Prior treatment	
Radiation to chest wall	9
Radiation to bone	8
Hormonal therapy or oophorectomy	17
Chemotherapy	
Cyclophosphamide, methotrexate, doxorubicin, and 5-FU	17
Vincristine	9
Vinblastine (bolus) and mitomycin	2
Melphalan, dacarbazine, or chlorambucil	3 (1 of each)
Initial dose of vinblastine (mg/m^2/day)	
1.2–1.3	4
1.4–1.5	10
1.7–1.8	3
No. of courses	
1	6
2	7
3–7	4

*Patients with cranial metastases were included only if they were in remission after cranial irradiation and if they had metastases elsewhere.

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[1] Received Feb 25, 1982; revised May 10, 1982; accepted June 16, 1982.

[2] Princess Margaret Hospital and Ontario Cancer Institute, Toronto, Canada.

[3] The Mississauga Hospital, Mississauga, Ontario, Canada.

[*] *Reprint requests to:* Ian F. Tannock, MD, The Princess Margaret Hospital, 500 Sherbourne St, Toronto, Ontario, Canada M4X 1K9.

confidence limits, 0–19%). The reason for the discrepancy between our results and those of Yap et al (1) is unclear. All of our patients had far-advanced and extensively treated disease but this was also true of the M. D. Anderson study: the mean number of prior chemotherapy regimens (2.8; range, 2–4) was less in our study than in that reported by Yap et al (1). We also include all patients entered in the study as evaluable for response, whereas four of 34 were excluded in the latter report. However, this policy does not account for the large difference in response rate between the two series (11 responses among 30 patients vs no responses among 17 patients) ($P < 0.01$, Fisher's exact test).

It is possible that vinblastine given by infusion might be more effective and less toxic when given to patients with metastatic breast cancer earlier in the course of their disease. However, since the treatment required either hospitalization or the use of an infusion pump and there are effective alternatives that can be administered to outpatients, we do not recommend vinblastine by infusion for treatment of metastatic breast cancer.

REFERENCE

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LETTERS

Fever Due to 6-Mercaptopurine

Fever can be caused by various drugs, including sulfonamides, iodides, thiouracils, barbiturates, and phenolphthalein-containing laxatives. The knowledge that chemotherapeutic agents can produce fever is crucial, since fever in cancer patients receiving chemotherapy is usually thought to be due to infection, consequently leading to broad spectrum antibiotic treatment. This is even more important in patients receiving aggressive chemotherapy for acute leukemia. Chemotherapeutic agents known to produce fever include bleomycin and cytarabine. 6-Mercaptopurine, an agent used in the treatment of acute leukemia, has to our knowledge been reported to produce fever only once (1).

CASE REPORT

A 24-year-old white female with terminal deoxynucleotidyl transferase-positive acute lymphocytic leukemia received remission induction treatment with doxorubicin, vincristine, and prednisone. She was not in complete remission after 5 weeks and was therefore switched to a combination of cyclophosphamide, cytarabine, and 6-mercaptopurine (100 mg orally daily taken for 3 weeks). One week after starting this combination she developed fever > 39°C. Since her granulocyte count was < 1000/mm^3, several cultures were taken and she started receiving three antibiotics; 3 days later miconazole was added to the regimen. All cultures were negative. The fever did not subside until the day after 6-mercaptopurine was discontinued, when she was still severely granulocytopenic. At this point the possibility of drug-induced fever was suspected. Following bone marrow recovery the patient was found to be in complete remission. Maintenance chemotherapy was initiated and consisted of 30 mg of methotrexate iv once per week and 100 mg of 6-mercaptopurine orally daily. About 1 hour after the first dose of 6-mercaptopurine, the patient developed fever > 39°C that lasted for about 6 hours. Following this episode, no abnormal laboratory findings, including results of liver function tests, could be detected. 6-Mercaptopurine was discontinued and 6-thioguanine was substituted, which was well-tolerated. The patient continued to be afebrile during the course of her maintenance treatment with methotrexate and 6-thioguanine.

Although in granulocytopenic patients fever of unknown origin is usually due to infection, fever due to chemotherapeutic agents may be another possibility.

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Richard Herrmann
Christian Manegold
Dieter Fritze

Medizinische Universitatsklinik
Bergheimer Str. 58
6900 Heidelberg, W Germany

April 22, 1982

Aminoglutethimide-Induced Thrombocytopenia

Hematologic toxicity secondary to aminoglutethimide, although uncommon, has been reported and is usually manifested as leukopenia[1] (1,2). Isolated thrombocytopenia secondary to this drug has occasionally been mentioned but few clinical details regarding this form of toxicity are available. We describe here a case of isolated thrombocytopenia that occurred after 4 weeks of therapy with aminoglutethimide. It resolved rapidly after discontinuation of the drug and recurred immediately upon reinstitution of drug therapy.

CASE REPORT

The patient, a 58-year-old white female, was hospitalized due to worsening low back pain secondary to breast cancer that was metastatic to the lumbosacral spine. She had recently undergone a course of radiation therapy to this region and had received a brief course of cyclophosphamide, methotrexate, 5-FU, and prednisone chemotherapy without substantial relief of her pain. Four weeks prior to admission, she began receiving 250 mg of aminoglutethimide orally four times per day and 100 mg of hydrocortisone every day in divided doses. On the day of admission (November 24, 1980) a cbc showed a hemoglobin of 12.4/100 ml, a hematocrit of 37%, a platelet count of 220,000/mm^3, and a wbc count of 4200/mm^3. The patient's drug regimen included 50 mg of hydrochlorothiazide given orally twice weekly, guaifenesin (Robitussin-DM) cough syrup as needed, aminoglutethimide, and hydrocortisone, all of which were continued.

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[1] Harvey HA. Personal communication, 1981.

Twelve days after admission (December 6, 1980), a cbc showed a hemoglobin of 12.5 g/100 ml, a hematocrit of 36.1%, a wbc count of 3400/mm^3, and a platelet count of 40,000/mm^3. Physical examination showed no petechiae, ecchymoses, or hepatosplenomegaly. Prothrombin and activated partial thromboplastin times were normal. All medications were then discontinued but the patient's platelet count continued to fall over the next 3 days to a nadir of 15,000/mm^3, with no significant change in her hematocrit or chronically borderline low wbc count. The patient refused to have a marrow aspirate performed in any area other than the posterior iliac crest, which had been previously irradiated. On December 6, 1980, an aspirate from that area was obtained but was hypocellular and nondiagnostic.

By 7 days later (December 13, 1980) the platelet count had risen to 155,000/mm^3. Aminoglutethimide and hydrocortisone were restarted since the thrombocytopenia was thought to be related to a possible recent viral illness or to be secondary to the hydrochlorothiazide. No other drugs were administered. A repeat platelet count 2 days later (December 15, 1980) was 50,000/mm^3, prompting discontinuation of the aminoglutethimide. The prothrombin and activated partial thromboplastin times were normal and the peripheral blood film was remarkable only for showing a decrease in the number of platelets. Over the next 9 days the platelet count progressively increased to 250,000/mm^3 and has been normal ever since, even after reinstitution of hydrochlorothiazide.

Hematologic toxicity due to aminoglutethimide has been reported infrequently, although one well-documented case of pancytopenia due to aminoglutethimide has been published (3). Occasional cases of agranulocytosis and/or thrombocytopenia thought to be secondary to this drug have been seen, but in some of these instances the relationship between the drug and the hematologic toxicity has not been clear-cut[1] (1). A recent paper by Asbury et al (2) reports a 4% incidence of thrombocytopenia in a group of 72 women treated with aminoglutethimide. However, no details regarding the thrombocytopenia were given in their paper.

When isolated thrombocytopenia first developed in this patient, its relationship to aminoglutethimide was not recognized, since thrombocytopenia is more commonly associated with the use of the thiazide diuretics. When thrombocytopenia developed rapidly following rechallenge with aminoglutethimide, a cause-and effect relationship was established. The rapidity of onset of thrombocytopenia in our patient is most consistent with a peripheral destructive mechanism, although an isolated immune or toxic suppression of megakaryocytes is also possible. Discontinuation of the drug after both initial therapy and rechallenge resulted in normalization of the platelet count within 7 days. Unfortunately, it was not possible to perform further studies that would have been helpful in elucidating the specific pathophysiologic mechanisms involved.

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Blair Ardman
Richard Rudders*

Department of Medicine
Hematology Oncology Division
New England Medical Center Hospital
171 Harrison Ave
Boston, MA 02111

May 24, 1982

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*To whom reprint requests should be addressed.

ANNOUNCEMENTS

Roswell Park Memorial Institute Continuing Education Programs

Buffalo, New York, October–December 1982

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on the newest methods in the diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute covering a different clinical specialty. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $25.00 registration fee for 1-day seminars and a variable fee for longer programs. The seminars are cosponsored by the Cancer Control Program of the Institute and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Cancer in the Elderly - An Interdisciplinary Perspective, *October 7–8, 1982* (Chairperson: Dr. Evan Calkins); Annual Meeting of the New York State Cancer Programs Association, *October 8–9, 1982;* Symposium on Dentistry and Cancer, *October 14, 1982* (Chairperson: Dr. Norman Schaaf); Computers in Medical Practice, *October 21, 1982* (Chairperson: Fil E. Vanvoris); Lung Cancer: Diagnosis and Therapy, *November 4, 1982* (Chairperson: Dr. Ronald Vincent); Contemporary Issues in Osteoporosis, *November 18, 1982* (Chairperson: Dr. Julian L. Ambrus); Comprehensive Care of the Advanced Cancer Patient, *December 9, 1982* (Chairperson: Dr. Edward Bockstahler).

For further information, contact: Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-4406).

Controversies in the Management of Early Breast Cancer

Los Angeles, California, November 17, 1982

This symposium, sponsored by the Hospital of the Good Samaritan, will take place at the Biltmore Hotel in Los Angeles, and will be acceptable for up to 6 hours of Category 1 credit toward CMA and AMA certificates in continuing medical education.

The fee, which includes a luncheon, will be $80 for practicing physicians, $45 for allied health professionals, and $35 for interns, residents, fellows, and retired physicians.

For further information, contact: Bonnie VanWaardenburg, The Hospital of the Good Samaritan, 616 S Witmer St, Los Angeles, CA 90017. Telephone: 213-977-2345.

Second Annual Bristol-Myers Symposium on Nutrition Research

Washington, DC, December 9–10, 1982

The role of nutrition in preventing and treating cancer will be examined by leading nutritional scientists at this symposium. Scheduled to be held at the Shoreham Hotel, the meeting is being organized by the Department of Nutrition Sciences, University of Alabama at Birmingham, and is sponsored by the Bristol-Myers Company and its Mead Johnson & Company subsidiary.

Outstanding scientists from a variety of disciplines will cover a range of topics, including vitamin deficiencies, fragile chromosomes, mononucleosis, lymphoma, nutritional "rescue," and primary chemoprevention with nutrition factors. Dr. Charles E. Butterworth, Jr, and Dr. Martha Hutchinson will cochair the 2-day event.

A major element in the program will be the presentation of the second annual Bristol-Myers Award for Distinguished Achievement in Nutrition Research. The recipient of the $25,000 cash award, selected by an independent panel of physicians and scientists, will be honored at a dinner on December 9.

For further information, contact: Ann D. Wyant or Kathryn R. Bloom, Bristol-Myers Co, 345 Park Ave, New York, NY 10154 (telephone: 212-546-4337).

Nominations For Sixth Annual Bristol-Myers Cancer Research Award

Nominations are now being accepted for the sixth annual Bristol-Myers award for distinguished achievement research. The award winner will be selected by an 11-member panel of judges from cancer research centers in the United States and Europe and will receive $50,000.

Nominations will be accepted from medical schools, free-standing hospitals, and cancer reseach centers until December 15, 1982. Only one nomination from each institution will be accepted.

For forms and further information, contact: Secretary, Awards Committee, Bristol-Myers Co, 345 Park Ave, Rm 43-38, New York, NY 10154.

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Cancer Invasion and Metastasis

Houston, Texas, March 1–4, 1983

This conference, the 36th Annual Symposium on Fundamental Cancer Research, will be sponsored by the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, and will be held at the Shamrock Hilton Hotel. The meeting will consider recent developments in the biology of tumor metastasis formation and investigate the possible contributions of these developments to cancer treatment. Program topics include tumor cell properties and metastasis, tumor invasion, tumor heterogeneity, host factors and metastasis, and therapy of metastasis. Cochairmen are Drs. Garth L. Nicolson and Luka Milas.

For additional information, contact: Office of Conference Services, Box 18, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030, or telephone (713) 792-2222.

American Radium Society Meeting

Savannah, Georgia, April 5–9, 1983

The 65th annual meeting of the American Radium Society will be held at the Hyatt Regency Hotel in Savannah, Georgia. *For further information, contact:* Mrs. Sally Polek, Office of the Secretariat, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

Fourth Annual Meeting of the Society for Clinical Trials

St. Louis, Missouri, May 8–11, 1983

The sessions of this meeting will focus on the design, organization, management, and analysis of clinical trials. Topics of interest for contributed papers or poster sessions include but are not restricted to: new methodology and associated problems and controversies in the design, monitoring, and analysis of clinical trials; data banks and data management; group or community clinical trials; clinical trials in specialized clinical areas; clinical trial results and their dissemination; patient compliance (adherence); monitoring for adverse effects or side effects; and ethical issues. Abstracts must be received by January 1, 1983.

For further information, contact: Dr. Christian R. Klimt, Secretary, Society for Clinical Trials, Inc, 600 Wyndhurst Ave, Baltimore, MD 21210.

Fourth International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy

Shelburne, Vermont, June 22–24, 1983

This symposium, sponsored by the Vermont Regional Cancer Center and the Norris Cotton Cancer Center, will address new developments in the biology, chemistry, pharmacodynamics, toxicology, and clinical aspects of platinum and other metal complexes as they relate to cancer therapy. The program is being designed to encourage the efficient exchange of concepts and foster discussion for the development of new concepts in this important area.

The program will consist of six scientific sessions. Each session will include presentations by three invited speakers, the viewing of scientific posters prepared by symposium attendees, and open discussion of the posters.

It is anticipated that 100–150 investigators from North America and Europe will participate, and that the proceedings of the symposium will be formally published. Deadline for poster abstracts is December 31, 1982.

For further information, contact: Joan MacKenzie, Vermont Regional Cancer Center, 1 S Prospect St, Burlington, VT 05401. Telephone: (802) 656–4414.

Thirteenth International Congress of Chemotherapy

Vienna, Austria, August 28–September 2, 1983

The 13th International Congress of Chemotherapy will be held at the KogreBzentrum-Hofburg in Vienna, Austria. The scientific program will consist of main lectures, symposia and workshops, round table sessions, and special paper poster discussions.

The program will include antimicrobial as well as anticancer topics. Proceedings will be preprinted and will be available to all participants at the congress. The deadline for submission of manuscripts (ready for photoprinting) is April 1983; the deadline for announcing papers, including title, authors, and main points of results, is December 1982. The preliminary announcement of intention to present a paper should be communicated as soon as possible.

For further information, contact: Secretariat of the XIIIth International Congress of Chemotherapy, c/o Wiener Medizinische Akademie, Alser StraBe 4, A-1090 Vienna, Austria. Telephone: (0222) 42 13 38. Telex: Chemo-A 13/4743. Cables: Medacad Wien.

Malignant Lymphoma: Current Status and Prospects

Lugano, Switzerland, June 13–16, 1984

The Second International Conference on Malignant Lymphoma will be held at the Palazzo dei Congressi in Lugano, Switzerland, and will deal with all important approaches in the diagnosis and treatment of Hodgkin's and non-Hodgkin's lymphoma in children and adults.

Most lectures will be given by invited speakers. Selected controversial issues will be discussed by panels of leading experts. The program will include free presentations and a poster session. The deadline for receipt of abstracts is January 15, 1984.

For abstract forms and further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland.

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1982

cancer treatment reports

October 1982
Volume 66
Number 10
Pages 1791-1888

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

HE 20. 3160:

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Supplied free of charge: 250 reprints (50 reprints for letters to the Editor) will be sent to the senior author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

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1887 ANNOUNCEMENTS

Doxorubicin-Vincristine Therapy for Wilms' Tumor: A Pilot Study[1,2]

Bruce Camitta,* Larry Kun, Marvin Glicklich, Herbert Oechler, Stuart Adair, and Donald Pinkel[3]

Doxorubicin plus vincristine chemotherapy was given to 31 children following nephrectomy for Wilms' tumor. Radiation therapy was used as indicated. Disease-free survival by stage is: eight of nine patients (stage I), eight of nine (stage II), nine of ten (stage III), and two of three (stage IV). Median follow-up of survivors is 28 months (range, 2–67); for all but four patients, follow-up is > 12 months. Two of the three stage I–III failures occurred in children with unfavorable histologies; the third failure was due to fatal anthracycline cardiomyopathy. Lowering the maximal cumulative doxorubicin dose from 450 to 240 mg/m^2 did not increase failures. Doxorubicin-vincristine appears to be effective chemotherapy for Wilms' tumor. [Cancer Treat Rep 66:1791–1794, 1982]

Wilms' tumor is the most common renal tumor of childhood. Although local disease can be eliminated with surgery and irradiation, 50% of children develop metastases (primarily pulmonary). The occurrence of metastases despite control of the primary tumor suggests that microscopic metastatic deposits are present at the time of diagnosis. Only one-half of the children developing macroscopic pulmonary disease during therapy remain free of disease despite additional treatment (1,2). Nonpulmonary metastases have even worse prognostic import (2). Therefore, the goal of adjuvant chemotherapy in Wilms' tumors is to eliminate metastases before they become clinically apparent.

Early trials of chemotherapy for Wilms' tumor focused on dactinomycin. Eleven of 39 children (28%) with metastatic disease achieved complete or partial responses with this drug (3–5). In patients with nonmetastatic disease, an initial report suggested that dactinomycin increased 2-year disease-free survival to 70% (6). In a subsequent study (7), the combination of dactinomycin plus vincristine increased disease-free survival when compared to either agent alone.

We investigated the combination of doxorubicin plus vincristine for treatment of Wilms' tumor. This combination was chosen instead of adding doxorubicin to vincristine plus dactinomycin because: (*a*) complete or partial responses of metastatic Wilms' tumor are more frequent with doxorubicin (60%) or vincristine (54%) than with dactinomycin (3–5,8–17) and (*b*) doxorubicin and dactinomycin have overlapping toxic effects whereas doxorubicin and vincristine do not. Results in our series suggest that doxorubicin plus vincristine is an effective, well-tolerated therapy for Wilms' tumor and should receive broader clinical trial.

PATIENTS AND METHODS

From June 1976 to October 1981, 31 patients with Wilms' tumor were entered in the study. This represents all patients with Wilms' tumor seen during that interval, except for one child with stage IV disease whose parents refused therapy. Preoperative evaluation included chest X-rays, lung tomograms, iv pyelogram, liver-spleen scan, bone scan, cbc, BUN, creatinine clearance, and liver function tests.

To maximize exposure, the involved kidney was removed through a thoracoabdominal incision unless the tumor was small or located in the inferior pole. A lung biopsy was done if patients had a thoracoabdominal incision. A bone marrow aspirate or biopsy was done at surgery in all patients.

Tumors were staged according to the National Wilms' Tumor Study (NWTS) staging system (7). Pathologic review followed guidelines set by Lawler et al (18) and the NWTS group (19).

Chemotherapy began within 6–10 days of surgery.

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[1] Received Feb 24, 1982; revised May 6, 1982; accepted May 18, 1982.

[2] Supported in part by the MACC fund and by Public Health Service grants CA-17700 and CA-17997 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Midwest Children's Cancer Center, Milwaukee Children's Hospital, and the Departments of Pediatrics (*B. Camitta, S. Adair,* and *D. Pinkel*), Radiation Therapy (*L. Kun*), Surgery (*M. Glicklich*), and Pathology (*H. Oechler*) of the Medical College of Wisconsin, Milwaukee.

**Reprint requests to:* Bruce Camitta, MD, Midwest Children's Cancer Center, 1700 W Wisconsin Ave, Milwaukee, WI 53233.

Vincristine (1.5 mg/m^2) was given weekly × 6, then every other week for 22 more doses. In the original protocol, doxorubicin was given at Weeks 1 (60 mg/m^2) and 4 (30 mg/m^2), then every 4 weeks (60 mg/m^2) for six more doses. However, because of cardiac toxicity in one patient and concerns about doxorubicin-irradiation interactions in other tissues, doxorubicin doses were reduced to 30 mg/m^2; thus, most patients received a cumulative dose of 240 mg/m^2 administered in eight equal doses. Doses of chemotherapy occasionally were delayed but rarely were decreased for neurologic or hematologic toxic effects.

Radiation therapy initially followed the guidelines of Cassady et al (1). Patients with grossly resected tumors received 2400–2600 rad in 100–150-rad fractions to the "tumor bed" (the entire region of initial tumor involvement and adjacent retroperitoneal lymph nodes, including the full width of the vertebral column). After results of NWTS I and NWTS II were established, radiation therapy was omitted for patients with stage I disease (7,20). Preoperative or intraoperative intraperitoneal spill was treated by 1900–2000 rad in 100–150-rad fractions to the whole abdomen (blocking the uninvolved kidney at 1400 rad), then boosting the tumor bed to doses as above. Pulmonary metastasis was treated by 1650–1800 rad of whole-lung irradiation in 100–150-rad fractions followed by a 1200–1400-rad boost to isolated nodules.

Follow-up included physical examinations, chest X-rays (monthly for 2 years, then at increasing intervals), liver-spleen scan (at 12 and 24 months), bone scan (at 12 and 24 months), echocardiography, blood cell counts (monthly during chemotherapy), SMA-12 (every 3 months during chemotherapy), and creatinine clearance (at 3 and 12 months). Failure of therapy was recorded for relapse at any site or for therapy-related death.

RESULTS

Patient age and sex and tumor stage and histology were typical for patients with Wilms' tumor. These parameters and details of therapy are presented in table 1.

Among 28 patients with stage I–III disease at diagnosis, there have been two treatment failures (one stage I and one stage III). Both patients had tumor histologies associated with a poor prognosis (19) and died despite salvage therapy with dactinomycin and irradiation. However, two other patients with unfavorable histologies remain free of disease 31 + and 33 + months after the beginning of therapy.

Two of three patients with stage IV disease at diagnosis remain free of disease (10+ and 24+ months after the beginning of therapy). The one patient who failed developed brain metastases after 8 months of treatment. After initially responding, the brain lesions became refractory to further irradiation and chemotherapy. At autopsy, there was no evidence of extracranial tumor.

Despite careful cardiac monitoring, one child developed congestive heart failure and died. Her cumulative doxorubicin dose was only 445 mg/m^2, but the abdominal radiation port included a portion of her heart. After the maximum allowable cumulative doxorubicin dose was lowered to 240 mg/m^2, no other child developed cardiomyopathy. The only other significant toxic effect was intestinal obstruction in three patients, two of whom had received total abdominal irradiation. A similar incidence of this complication has been reported in young children receiving extensive postoperative irradiation plus chemotherapy (21). Possible potentiation of radiation by doxorubicin in our cases cannot be ruled out.

DISCUSSION

Dactinomycin has been considered to be the mainstay of adjuvant chemotherapy for Wilms' tumor. A 70% (37 of 53 patients) 2-year disease-free survival rate was reported by Farber (6). However, there were no concurrent controls, and disease stage was not clear. In NWTS I, the 2-year disease-free survival rate following dactinomycin plus tumor bed irradiation was 83% for stage I and 57% for stages II + III (7). In an International Society of Pediatric Oncology (SIOP) study, the 2-year disease-free survival rate with dactinomycin plus tumor bed irradiation was 56% for stage I–III patients (22). Furthermore, although multiple courses of dactinomycin resulted in greater disease-free survival rates than a single course (72% versus 43%, respectively) in an early study (23), this was not confirmed in the larger SIOP study (58% with multiple courses versus 54% with a single course) (22). On balance, dactinomycin appears to increase disease-free survival by only 10% when added to surgery plus irradiation therapy for stage I–III Wilms' tumor (24).

Vincristine is more active than dactinomycin against metastatic Wilms' tumor (4,14–17). However, in NWTS I, the 2-year disease-free survival rate for stage II–III disease was 55% with vincristine and 57% with dactinomycin (7). In another study, all patients with stage I–III Wilms' tumor received one course of dactinomycin and were then randomized to receive further dactinomycin or vincristine (25). The 2-year disease-free survival rate was 54% with dactinomycin and 79% with vincristine ($P = 0.008$). Finally, when vincristine plus dactinomycin was used for stage II–III patients, the long-term disease-free survival rate was 81% in NWTS I and 65% in NWTS II (70% for both studies combined). One possible interpretation of these data is that vincristine and dactinomycin act synergistically against Wilms' tumors. Alternatively, dactinomycin may be relatively inactive, the above results reflecting primarily vincristine activity.

TABLE 1.—Patient characteristics, therapy, and clinical outcome

Patient No.	Age (yrs)	Sex	Stage (NWTS)*	Histology†	Tumor bed (other) radiation therapy‡	Total doxorubicin dose (mg/m²)§	Mos of continuous remission	Outcome, other
1	5	F	I	PD	+	421	71 +	
2	14	M	I	WD	+	357	66 +	
3	3	M	I	MD	+	270	60 +	
4	3	F	I	MD	0	240	49 +	Hemihypertrophy
5	2	M	I	Clear cell, PD	+	[240]	[21]	Bone metastasis, died (44 mos)
6	2	M	I	WD	+	240	38 +	
7	3	F	I	MD	0	240	32 +	
8	1	M	I	WD	0	240	17 +	
9	1	F	I	WD	0	240	7 +	Uncle had Wilms' tumor
10	5	F	II	MD	+	[445]	[10]	Fatal cardiomyopathy; no tumor at autopsy
11	8	M	II	MD	+	288	59 +	
12	2	F	II	MD	+	240	46 +	Concomitant renal adenoma
13	1	M	II	MD	+	240	37 +	
14	5	F	II	WD	+	240	34 +	
15	4	F	II	Clear cell, PD	+	240	31 +	
16	4	F	II	WD	+	240	31 +	
17	4	M	II	MD	+	240	26 +	
18	1	F	II	MD	+	240	21 +	Multifocal intrarenal tumors
19	3	M	III R	PD	+ (WA)	297	57 +	
20	10	M	III ¶	Anaplastic, PD	+ (WA)	[205]	[8]	Lung metastases; died (23 mos)
21	6	F	III S	MD	+ (WA)	240	43 +	
22	11	F	III S	Anaplastic, PD	+ (WA)	240	33 +	Rhabdoid elements
23	5	F	III S	MD	+ (WA)	240	32 +	
24	2	M	III	MD	+	240	29 +	
25	1	F	III S	PD	+ (WA)	240	17 +	Nodes, positive
26	1	M	III R	MD	+ (WA)	240	16 +	
27	3	M	III	WD	+	240	9 +	Local spill
28	3	F	III R	MD	+ (WA)	240	7 +	Invasive
29	8	F	IV S	PD	+ (WA, WL)	240	[8]	Brain metastases; died (19 mos), CNS tumor only at autopsy
30	5	M	IV	WD	+ (WL)	240	24 +	
31	2	F	IV	WD	+ (WL)	240	10 +	

*S = operative spill; R = preoperative rupture.

†WD, MD, PD = well, moderate, or poor tubular differentiation and not clear cell or anaplastic. *See* refs 18 and 19 for details.

‡WA = whole abdomen; WL = whole lung.

§Dose at failure is shown in brackets.

¶Inoperable. Biopsied elsewhere. Received doxorubicin (× 1) and vincristine (× 2) at Milwaukee Children's Hospital before resection.

Doxorubicin also is more active than dactinomycin against metastatic Wilms' tumor (8–13). In NWTS II, when doxorubicin was added to vincristine plus dactinomycin for stage II–III tumors, long-term disease-free survival rates increased from 65% to 80% (20). In our study, 17 of 19 (89%) children with stage II–III disease remain alive and disease-free after treatment with doxorubicin plus vincristine. These data suggest that doxorubicin is more effective than dactinomycin for adjuvant therapy of Wilms' tumor. Alternatively, our excellent results might be due in part to a more effective time-dose schedule of doxorubicin and vincristine in addition to substitution of doxorubicin for dactinomycin per se. More intensive dactinomycin-vincristine is being compared to doxorubicin-dactinomycin-vincristine in NWTS III. Given equivalent antitumor efficacy and short-term toxicity, a regimen not utilizing doxorubicin would be preferable because of less potential long-term cardiac toxicity.

In NWTS II, the disease-free survival rate of children with stage I disease was 90% after 6 or 15 months of dactinomycin-vincristine. One-half of the relapses occurred in patients whose tumors had unfavorable histologies (20). Despite similar results using doxorubicin-vincristine in our study, dactinomycin-vincristine is preferable for children with favorable-histology stage I disease, because of decreased potential cardiac toxicity.

Too few patients with stage IV disease or unfavorable histologies were treated in this study to make meaningful comparisons with previous regimens. Current therapy may cure 40%–60% of children presenting with stage IV disease (1,2) and 30%–40% of children whose tumors have unfavorable histologies (2,19,20). Doxorubicin-vincristine as utilized in this pilot study deserves further trials in children presenting with these types of Wilms' tumors.

Our data suggest that doxorubicin may be more effective than dactinomycin in the treatment of Wilms' tumor. At a maximum cumulative doxorubicin dose of 240 mg/m^2, clinically apparent cardiac toxicity is extremely rare (26). However, even low doses of doxorubicin may cause microscopic cardiac damage (27). The long-term consequences of these subclinical lesions are not known. Therefore, caution is suggested before results in this study are applied to larger groups of patients with Wilms' tumor.

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Invited Comment on the Milwaukee Pilot Study of Doxorubicin-Vincristine Therapy for Wilms' Tumor

Dr. Camitta and colleagues (1) report very impressive results in a study of Wilms' tumor in a single institution. Thirty-one patients representing all stages of the disease, four of them with unfavorable histologic features, were treated with a regimen that included doxorubicin (ADR) and vincristine (VCR). Twenty-seven of the 31 children survive for periods ranging from 2 to 67 months (median follow-up time, 28 months). Two of the four failures occurred in patients with tumors of unfavorable histology, one died of cardiotoxicity, and one had metastases at diagnosis.

The authors present a brief but complete review of the literature concerning ADR therapy for Wilms' tumor, present their rational for the use of this agent (which they consider to be superior to dactinomycin [AMD]), and attribute the good results reported to the use of ADR. However, there are other possible explanations for the better results in these patients.

Camitta and his colleagues gave both ADR and VCR more frequently than they have generally been given in the large cooperative studies. Thus, the Milwaukee investigators administered VCR weekly for 6 weeks, and then every other week for 22 additional doses. This is to be contrasted with the way VCR was used in the first and second National Wilms' Tumor Studies (NWTS I and II) and by the International Society of Pediatric Oncology, where it was given every 3 months during the maintenance phase. The United Kingdom Medical Research Council results, mentioned by Camitta and colleagues, found that VCR every other week gave results superior to those with the 3-month schedule used in NWTS I. AMD, too, was given every 3 months in NWTS I and II, rather than on a monthly schedule such as that employed in Milwaukee. Green et al (2) found that AMD appeared to be more efficient in leukemic patients when given monthly than when given in the more traditional 3-month cycles. Similar observations were recorded by Green (3) for children with solid tumors given AMD, VCR, and cyclophosphamide in relatively high-dose pulses.

Therefore, it is equally plausible that the good results reported by Camitta et al are secondary to a more efficient use of effective agents, rather than to the ADR itself. This hypothesis is being put to the test in the third NWTS, where both AMD and VCR are being given more frequently than before (4), although the schedule as well as the drugs are different from those used by Camitta et al. Preliminary data from NWTS III do not suggest any major difference between AMD and VCR used more intensively versus the three-drug regimen (AMD + VCR + ADR) that gave the best overall results in NWTS II.

Thus, while the Milwaukee results are impressive, it remains to be shown that it is the ADR rather than the scheduling differences that are responsible. These considerations would matter relatively little if it were not for the fact that even modest doses of ADR are associated with demonstrable myocardial damage (5). Thus, while the total dose of ADR used in Milwaukee seems to be safe on the basis of short-time observations, the long-term effects—measured in decades and scores of years in surviving children—are unknown and are reasons for concern. Indeed, it is this latter consideration which led the NWTS group to test the more intensive AMD + VCR regimen against the one that included ADR as well. The object was to avoid the use of ADR as a routine in these young children if it could be shown that elimination of the drug did not significantly affect relapse-free survival.

Camitta et al are correct in stating that the objective in pediatric oncology is to obtain relapse-free survival in as many patients as possible, but the cost in terms of toxicity in achieving such "coverage" needs to be put in the balance. NWTS II has shown that 88% of group I children survive relapse-free 2 years beyond the date of surgery when they are given AMD + VCR (without radiation therapy) postoperatively. Of children with group II or III Wilms' tumor of favorable histology, 70% survive free of disease ≥ 2 years after postoperative irradiation, AMD, and VCR. Addition of ADR to the regimens for these patients means that seven or more of ten children with locoregional tumors of favorable histology would be given the cardiotoxin unnecessarily. Camitta and his colleagues are therefore well-advised when they caution against indiscriminate trials of ADR in patients with Wilms' tumor.

The Milwaukee data should encourage investigators to try different schedules of drugs of known or marginal effectiveness, not only in Wilms' tumor but in other tumor systems as well. The report is also of interest because the total treatment time is shorter than that in several large studies. It may well be that the more efficient use of effective chemotherapeutic agents not only will give better results but also will shorten the overall time that treatment must be administered to patients. This is a gain of considerable importance in itself.

Finally, the article is important in another way. It demonstrates that there is ample room for carefully planned and well-conducted clinical investigations in single institutions. This pertains even in the study of neoplasms that appear to be dominated by cooperative clinical trials such as the NWTS and studies by the International Society of Pediatric Oncology and the Medical Research Council.

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Giulio D'Angio

Children's Hospital of Philadelphia
34th and Civic Center Blvd
Philadelphia, PA 19104

July 19, 1982

Treatment of Advanced Prostate Cancer With Cyclophosphamide, Doxorubicin, and Methotrexate[1,2,3]

Marc J. Straus,[4,*] Jody P. Fleit,[4,5] and Constance Engelking[4,6]

Twenty-three patients with advanced prostate cancer who had failed previous hormone therapy were treated with cyclophosphamide, doxorubicin, and methotrexate on a 3-week course. Of the 22 evaluable patients, over one-half had poor performance status, increased acid and alkaline phosphatase levels, and pain. Parameters which improved in > 50% of cases included acid and alkaline phosphatase levels, pain, performance status, and measurable lesions (lung and soft tissue). Initial parameters associated with a significantly decreased survival were age > 66 years, increased pain, poor performance status, and increasing alkaline phosphatase. Prior radiation therapy was associated with increased drug toxicity, lower doses of chemotherapy, and decreased survival (not significant). There was a significant relationship between the degree of improvement of acid phosphatase, alkaline phosphatase, pain, and performance status to increased survival. Three categories of response were defined based on these parameters. The mean survival of seven patients with partial response (106 weeks) is significantly longer than that of seven with measurable response (57 weeks) and eight with no response (26 weeks). Four patients had severe leukopenia and one died of sepsis. These results compare favorably with previous reports of chemotherapy treatment of advanced prostate cancer. [Cancer Treat Rep 66:1797–1802, 1982]

Only recently has there been an emphasis on the treatment of advanced prostate cancer with combination chemotherapy (1). Drugs with activity include cyclophosphamide (Cytoxan; Cyt), doxorubicin (Adriamycin; Adr), 5-FU, and methotrexate (MTX) (1,2).

Several studies (3–6) have included the combination of Cyt and Adr because of its efficacy in other solid tumors (7). There is evidence that Cyt is more effective when given in high intermittent doses than as daily oral therapy (8,9). In this study, Cyt and Adr were administered on Day 1 followed by four biweekly doses of MTX beginning 8 days later. There are cytokinetic data in other solid tumors (10,11) to suggest an advantage to administration of an S-phase drug like MTX when recruitment of cells into DNA synthesis has occurred. A similar schedule of therapy was shown to be advantageous for lung cancer (10).

A standard nomenclature for response criteria was developed by the National Prostatic Cancer Project (2). However, many recent studies have defined their own criteria (3,4,12,13), in part due to the need for categories that seem more relevant to results now being achieved. Advanced prostate cancer has important features such as a high percentage of positive bone scans and acid and alkaline phosphatase levels, but few patients have measurable lesions.

In the present study we have analyzed a series of pretreatment variables and their relation to survival. Several parameters have been serially studied and related to response and survival. The Cyt, Adr, and MTX (CAM) regimen has induced a high percentage of remissions and improved survival and, as a consequence, it has been possible to determine several parameters associated with a better prognosis.

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[1] Received May 4, 1981; revised Aug 19, 1981; accepted July 21, 1982.

[2] Supported in part by the Dr. "I" Fund Foundation, New York, NY.

[3] Presented in part in the Proceedings of the American Society of Clinical Oncology, Washington, DC, Apr 1978.

[4] Division of Neoplastic Diseases, Department of Medicine, New York Medical College, Valhalla.

[5] *Present address:* Womens and Infants Hospital, Providence, RI.

[6] We acknowledge the statistical analysis by Daniel Billet and Marc Serber.

Reprint requests to: Marc J. Straus, MD, 311 North St, White Plains, NY 10605.

MATERIALS AND METHODS

From April 1976 to December 1979, 23 patients with stage D prostatic carcinoma were entered in this protocol. One patient who developed a spinal cord compression prior to receiving the second course of therapy was excluded from analysis. Patients were excluded with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 4, or a cardiac history or ECG contra-indicating the use of Adr. Most patients had received previous treatments (table 1). Patients who had had a prior orchiectomy and/or treatment with hormones had evidence of disease progression. No patient had received previous chemotherapy. Of the 12 patients who had had prior radiation, eight had received 5000–8000 rads to the prostate and pelvis, and one of these had also received radiation to the peri-aortic area. Four patients had had radiation to sites of bony metastases: hip, ribs, and spine.

The data of the 22 evaluable patients were analyzed as of April 1981. The mean age was 69 years (range, 52–83). All patients were evaluated with: complete history and physical examination, cbc and platelet count, blood chemistries, serum prostatic acid phosphatase, urinalysis, chest X-ray, IVP, and bone scan. Most patients had a liver scan, a bone marrow biopsy and aspirate, and a serum carcinoembryonic antigen (CEA).

The ECOG PS scale (0–4) was used. Pain levels and urinary obstructive systems were graded on a 4-point (0–3) scale of increasing severity.

Histology sections were classified as well, moderately, or poorly differentiated.

Measurable chest X-ray and subcutaneous lesions were determined by caliper, the longest diameter times its perpendicular.

Physical evaluation, blood chemistries, and analysis of subjective parameters were repeated every 3 weeks. Chest X-rays were repeated every 6–9 weeks, if abnormal. The bone and liver scans and IVP were repeated every 3 months, if abnormal. Bone marrow determinations were not repeated. The liver scan was accepted as abnormal if there were distinct multiple defects.

TABLE 1.—Previous treatment of 22 patients receiving CAM treatment for prostate cancer

Previous treatment	No. of patients
Prostatectomy	4
TURP*	16
Orchiectomy	15
Radiation	12
Hormonal	17

*Transurethral resection of the prostate.

All patients began treatment with a normal wbc and platelet count and creatinine of < 1.6 mg/100 ml. Treatment consisted of 600 mg/m^2 iv of Cyt and 40 mg/m^2 iv of Adr on Day 1, and 15 mg/m^2 orally of MTX on Days 9, 13, 16, and 20. The courses were repeated every 3 weeks and the doses were adjusted as shown in table 2. After an episode of severe leukopenia following the first treatment in two patients who had had prior radiation, initial treatments were reduced to two-thirds for this group.

The parameters that were determined were divided into objective (table 3) and subjective (table 4) parameters. For the abnormal blood tests, a complete response (CR) was defined as normalization of the laboratory value. A partial response (PR) was defined as a 50% decrease in an abnormal value but the decreased value remained above normal limits. A CR for measurable lesions, liver scan, or bone scan required normalization. A PR for measurable lesions and liver scan defects was a 50% decrease in the product of the largest diameter and its perpendicular.

For subjective parameters, a CR represented normalization, eg, to PS 0. A PR was a clear improvement of at least one grade.

In the overall analysis, establishing definitions of response is more complicated. In this and other studies, an abnormal bone scan or enlarged prostate rarely nor-

TABLE 2.—Dose modifications of CAM

Blood count (cells/mm^3) on Day 1 of new treatment course		Dose modifications		
Wbcs	Platelets	Cyt	Adr	MTX
≥ 4000	$\geq 100{,}000$	Increase 10%	Increase 10%*	No change
3000–4000	$\geq 100{,}000$	No change	No change	No change
2000–3000	$\geq 100{,}000$	Give $^2/_3$ dose	Give $^2/_3$ dose	No change
≥ 3000	75,000–100,000	No change	Give $^2/_3$ dose	Give $^2/_3$ dose
≥ 3000	50,000–75,000	Give $^2/_3$ dose	Omit†	Omit
2000–3000	$\geq 50{,}000$	Give $^1/_3$ dose	Omit	Give $^1/_3$ dose
≤ 2000	$\leq 50{,}000$	Omit	Omit	Omit

*Adr should not exceed 60 mg/m^2.

†After dosage omission, resume next dose at $^2/_3$ of offending dose of wbcs ≥ 3000/mm^3 and platelets $\geq 100{,}000$/mm^3.

Table 3.—Objective parameters of 22 patients receiving CAM treatment for prostate cancer

Parameter	No. of patients tested	No. abnormal	No. of — CR	PR	% response
Measurable*	22	7	5	2	100
Alkaline phosphatase	22	19	9	5	74
Acid phosphatase	22	12	3	4	58
CEA	18	9	2	3	56
Liver scan	18	5	1	1	40
Large prostate	22	9	0	3	33
Bone scan	22	22	1	0	5
Bone marrow	13	6	—	—	—

*8 lesions in 7 patients: 3 lung nodules, 2 abdominal masses, 1 node, 1 subcutaneous lesion, 1 retro-orbital mass. Patient with 2 lesions, both responding, was considered a PR.

malized. We therefore defined a PR as complete normalization of all abnormal objective parameters except an abnormal bone scan or enlarged prostate. A measurable response (MR) was defined as a 50% improvement in all objective parameters, except bone scan and enlarged prostate. All other patients had no response (NR). These definitions provide the maximum consistency with definitions recently used by others (1,3–7). We have attempted in effect to define three categories of overall response. The labels PR, MR, and NR are not ideal descriptors.

RESULTS

Twenty patients were white and two were black. The majority of patients had previously received several types of treatment (table 1). Ten patients had PS 0 or 1, and 12 had PS 2 or 3.

The parameters and responses are shown in tables 3 and 4. Bone scans were abnormal in all patients. Pain, abnormal PS, and an elevated alkaline phosphatase each occurred in 19 of 22 patients. Acid phosphatase was elevated in 55%.

There were eight measurable lesions noted in seven patients which included a retro-orbital mass and lung nodule in one patient. There were two other lung lesions, one subcutaneous lesion, one node, and two abdominal-pelvic masses. In all seven patients there was a response: five CR and two PR. The patient with retro-orbital disease had marked proptosis that normalized and was considered a PR. Alkaline phosphatase objectively improved in 74% of patients and acid phosphatase improved in 58%. Bone scan objectively improved in only one patient. There was significant improvement of pain in over three-fourths of the patients and an improved PS in over one-half.

Survival data according to response are shown in table 5. The overall mean survival was 61 weeks. The median survival was 47 weeks, with two patients alive. The patients with PR lived significantly longer than those with MR ($P < 0.001$) or NR ($P < 0.001$), and those patients with MR in turn lived significantly longer than those with NR ($P < 0.05$).

There was no apparent influence of histologic grade on survival. The type of previous hormonal treatment or surgery did not influence survival.

The factors which may have influenced survival are shown in table 6. Patients ≤ 66 years old survived longer than older patients ($P < 0.01$). An analysis of variance demonstrated a significant trend of increasing age and decreased survival ($P < 0.01$). Patients with little or no pain (grades 0 or +1) survived longer than those with grades +2 or +3 ($P < 0.10$). Prior radiation was associated with reduced survival ($P = 0.13$), and patients who had PS 0 or 1 survived longer than those who had PS 2 or 3 ($P < 0.05$).

Factors which did not influence survival were initial CEA, abnormal liver scan, measurable tissue lesions, urinary obstructive symptoms, and positive bone marrow. Prior surgery had a negative effect, but it was not significant.

An analysis of variance demonstrated no significant relationship between initial acid phosphatase and sur-

Table 4.—Subjective parameters of 22 patients receiving CAM treatment for prostate cancer

Parameter	No. abnormal	No. of — CR	PR	% response
Pain	19	9	6	78
PS	19	6	5	58
Urinary obstruction	11	4	1	45

TABLE 5.—CAM treatment of prostate cancer: response

Response	No. of patients		Survival in wks	
	Total	Alive	Mean (range)	Median
PR	7	1	106 (41–258+)	90
MR	7	1	57 (17–108)	57
NR	8	0	26 (15–45)	21
Total	22	2	61 (15–258+)	47

vival, although the two survivors are in the normal acid phosphatase group. Few patients had normal alkaline phosphatase values. There was a significant trend of increasing values and poorer survival ($P < 0.05$).

The extent of response of acid phosphatase, alkaline phosphatase, pain, and PS was related to survival; for these parameters, the survival was normal > CR > PR > NR. For alkaline phosphatase and PS, this trend of decreasing survival was significant at $P < 0.01$ and $P < 0.001$, respectively. Survival of CR was significantly greater than NR for all groups and greater than PR for pain and PS. Improvements of measurable lesions, CEA, scans, and urinary obstruction were too infrequent for a similar analysis.

The average survival from diagnosis to beginning this treatment was 144 weeks (range, 2–596). There was no relationship between this pretreatment interval and posttreatment survival. The average survival from diagnosis to treatment for patients with PR, MR, and NR was 101, 155, and 171 weeks, respectively (not significant). Similarly, there was no relationship to any pretreatment parameters.

This treatment resulted in a nadir wbc count < 1000/mm^3 in four patients, sepsis in three patients, and death in one patient. All four patients had received prior radiation.

To determine the possible effect of pretreatment parameters on drug dose and leukopenia, an analysis was made of the nadir leukopenia and doses of Cyt and Adr during the third course of treatment. A comparison of the lowest wbc count for each patient would be biased by the fact that patients who received more courses and survived longer had greater opportunity for lower blood cell counts. By the third course of treatment, patients started at lower initial drug doses due to previous radiotherapy could have been dose-incremented to full doses if blood cell counts permitted. Seventeen patients were evaluable. There were no statistically significant differences in nadir wbc count or dose of Cyt and Adr based on pretreatment parameters. Patients who had had previous radiation had a lower wbc count ($P < 0.10$) and received less Cyt ($P < 0.10$) than patients with no previous radiation. There were too few patients to make a distinction between those who received a major course of radiation to the pelvis (≥ 5000 rads) and those whose radiation was to metastatic sites only. There were no differences based on categories of response.

Mucositis occurred frequently and MTX was decreased progressively until the mucositis no longer occurred. Alopecia was universal. Adr was discontinued in one patient who developed early congestive heart failure after eight courses. The patient responded to

TABLE 6.—CAM treatment of prostate cancer: relation of age, pain level, previous radiation, and PS to survival

Parameter	No. of patients	No. alive	Survival in wks	
			Mean	Median
Age in yrs				
≤ 66	7	1	103	95
67–72	8	1	49	48
≥ 73	7	0	33	22
Pain level				
0– +1	8	2	94	81
+2	6	0	44	48
+3	8	0	41	32
Previous radiation				
No	10	2	81	50
Yes	12	0	44	37
PS				
0, 1	10	2	87	58
2, 3	12	0	37	32

diuretics and digitalis. One patient died of a myocardial infarction. The other deaths appeared to be related to the cancer.

DISCUSSION

In order to devise meaningful criteria for the evaluation of response in prostate cancer one must be able to follow various parameters serially, and the treatment must be relatively effective. Yagoda et al (13) discussed the advantages of selecting patients with measurable disease for study. In prostate cancer, patients with measurable lesions may not be a representative group. In this study 32% of patients had measurable lesions, all of whom responded to chemotherapy.

We divided the parameters into objective and subjective categories. Other studies have distinguished between direct and indirect parameters (4). Both systems are at best artificial. The important issue is to determine the prognostic value of the initial determination and the relation of a change in the parameter to survival (14).

The prostate size is not generally a useful parameter since so many patients undergo prior transurethral resection of the prostate and/or radiotherapy. The bone marrow is frequently positive but serial testing may be inconclusive.

Aside from a minority of patients with measurable or evaluable lesions, the study of response is restricted primarily to chemical, X-ray, and subjective measurements.

We defined changes of parameters in standard fashion, ie, CR = normalization. Our group of patients had a particularly poor prognosis, having all failed previous hormone therapy and 12 of 22 having PS 2 or 3. Typically, bone scans were positive and pain was frequent and often severe.

Our analysis indicated that younger patients have a significantly better prognosis (table 6). This is in accord with Berry et al (15) but not with Yagoda et al (13) or Eagan et al (12). The initial PS in our study was significantly related to survival (table 6). This has been noted by most investigators (13,15). Ihde et al (4) restricted their study to ambulatory patients.

Similarly, we found that the initial value for alkaline phosphatase correlated inversely with survival but the correlation for acid phosphatase was not significant. Berry et al (15) and Johnson et al (14) documented the significance of acid phosphatase in larger series.

We found that patients with previous radiation had appreciably decreased survival times. This relationship was noted by Schmidt et al (2) and DeWys et al (16) and as in our study seems to be due to reductions in chemotherapy dose in patients with prior radiation. In Berry's study (15) there was no relationship between prior radiation and survival. However, the chemotherapy used caused little myelosuppression.

Other initial parameters which may be associated with a poorer prognosis include: positive bone marrow, elevated CEA or LDH, decreased hemoglobin, prior surgery, poorly differentiated tumor, and positive liver scans (2,14,15,17,18). In our study of 22 patients these factors were not significant, which may in part reflect inadequate numbers of cases.

We found that improvement in acid or alkaline phosphatase, pain level, or PS significantly correlated with a better survival. Johnson et al (14) noted a similar correlation for acid phosphatase and pain but not for PS. Bone scans are not useful in the assessment of response since responses are as yet rarely, if ever, noted.

An overall assessment of response can be made once individual parameters are analyzed. We have been able to define three separate categories of overall response. In this study we defined PR, MR, and NR as stated. This is not unlike the approach of Ihde et al (4). In this study, the average survival of patients determined to have the best response (PR) was > 2 years compared to just > 1 year for MR patients and $^1/_2$ year for NR patients. Other definitions of response have proved useful (2–4,13). With further improvement in treatment results more exact criteria will be possible.

In this study, three drugs were used in a moderately toxic regimen. Many investigators heretofore have been especially leery of using doses of drugs in this disease which are comparable to those used in other diseases. Nevertheless, good response rates in this disease as in most other solid tumors will probably require adequate levels of drugs.

The response rate and survival in this study compare quite favorably with previous reports of combination chemotherapy (1). The overall survival exceeded 1 year, with approximately one-third of the patients living 2 years.

In most reports, patients previously treated with hormones survive < 30 weeks. Several recent reports have included the use of Cyt and Adr. Izbicki et al (3) reported a 17- and 40-week survival for nonresponders and responders, respectively. In a study restricted to ambulatory patients, the overall survival was 11 months (4). In a third study, average survival was not given (5). In this study, the survival of ambulatory patients was 20 months. Single agents have been far less effective.

The apparent improved results achieved may be due to somewhat higher drug doses. In addition, the use of the cycle-specific drug MTX may be important. In lung cancer the response rate is approximately doubled by addition of MTX in a similar schedule following pulse Cyt (19). MTX adds little to the hematologic toxicity as given, but does cause mucositis.

It is likely that further progress in prostate cancer will be made using combinations of drugs with the same vigor and fundamental designs that have been successful in other cancers. It will be important to develop a uniform nomenclature of response so that reports will be comparable. In the meantime, detailed testing and reporting of results will help elucidate the directions for further studies.

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Treatment of Advanced Colorectal and Gastric Adenocarcinomas With 5-FU Combined With High-Dose Folinic Acid: A Pilot Study[1]

David Machover,* Léon Schwarzenberg, Emma Goldschmidt, Jean Marc Tourani, Bernard Michalski, Marcel Hayat, Thierry Dorval, Jean Louis Misset, Claude Jasmin, René Maral, and Georges Mathé[2,3]

Thirty patients with advanced colorectal adenocarcinoma and five with advanced gastric adenocarcinoma were studied; all had measurable tumor. The treatment used was based on biochemical and cell culture studies which have demonstrated that an excess of intracellular reduced folates is necessary to provide optimal inhibition of thymidylate synthetase and to increase the cytotoxic effect of fluorinated pyrimidines. The treatment comprised 5-FU (370–400 mg/m^2/day) and high-dose folinic acid (200 mg/m^2/day) given simultaneously for 5 consecutive days, with a 21-day interval between treatments. Of the 30 patients with advanced colorectal carcinoma, 16 had not been previously treated with cytostatic agents and 14 were resistant to previous chemotherapy with 5-FU given either as a single agent or combined with other drugs. The response rates, both complete and partial, were 56% and 21% in the previously untreated and the previously treated patients, respectively. The five patients with advanced gastric adenocarcinoma were previously untreated; three achieved a partial response. The overall toxicity was acceptable. The most prominent side effect was oral mucositis; myelosuppression and diarrhea were less prevalent. Severe toxicity, when present, was prevented in subsequent courses by a 30-mg/m^2 reduction in the daily dose of 5-FU. We conclude that in these types of tumors, folinic acid at high doses increases the effectiveness of 5-FU given simultaneously and renders some colorectal tumors, previously resistant to 5-FU, sensitive to this drug. [Cancer Treat Rep 66:1803–1807, 1982]

The use of 5-FU as a single agent in the treatment of advanced colorectal and gastric adenocarcinomas has been disappointing; responses, generally of short duration, have been observed in 20% of the patients with colorectal carcinoma and in 25% of the patients with gastric carcinoma, but there has been no proven increase in the overall survival rate (1–3).

The rationale of the clinical trial reported here is based on the results of biochemical and cell culture studies performed with various cell lines (4–7), demonstrating that an excess of intracellular reduced folates is necessary for optimal inhibition of thymidylate synthetase (TS) and for an increased cytotoxic effect of fluorinated pyrimidines. It was our aim to enhance the activity of 5-FU in colorectal and gastric adenocarcinomas by simultaneous administration of folinic acid (FA) in high doses (8).

PATIENTS AND METHODS

Patients entered in this study had histologically confirmed colorectal or gastric adenocarcinomas; all had metastatic disease.

Patients with colorectal adenocarcinoma.—Thirty-five patients were entered in the study; five were excluded because their tumors could not be measured accurately. The 30 evaluable patients had measurable disease; 23 had histologically proven metastases, and seven had metastatic tumors as evidenced by radiologic, ultrasonographic, and/or scintigraphic proce-

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[1] Received Dec 16, 1981; revised June 15, 1982; accepted July 12, 1982.

[2] Service des Maladies Sanguines et Tumorales (ICIG, INSERM U-50), Hôpital Universitaire Paul-Brousse, Villejuif, France.

[3] We thank Profs. H. Rappaport and P. Reizenstein and Dr. J. Gouveia for their support and constructive comments and Mrs. X. Benavente for preparation of the manuscript.

**Reprint requests to:* Dr. David Machover, Service des Maladies Sanguines et Tumorales (ICIG, INSERM U-50), Hôpital Universitaire Paul-Brousse, 14–16 Avenue Paul-Vaillant-Couturier, 94804 Villejuif, France.

TABLE 1.—Patient characteristics and response

Patient No.	Age (yrs)	Sex	Prior treatment with 5-FU*	Tumor site	No. of courses at evaluation	Response to therapy†	CEA titer before/after therapy (ng/ml)†	Response site	Duration of response or disease stabilization (days)
				Patients with colorectal adenocarcinoma					
1	50	F	—	Pelvis, vagina	9	CR	7.8/2.5	Pelvis, vagina	258+
2	58	M	—	Liver	9	PR	99/47	Liver	258+
3	59	M	—	Liver	7	PR	2.5/2.5	Liver	197+
4	55	F	—	Liver	8	PR	64/55	Liver	213+
5	50	M	—	Liver, lung (nodular)	8	PR	13/<2.5	Liver, lung	229+
6	58	F	—	Liver, abdominal tumor	6	PR	11.7/5.4	Liver, abdomen	136+
7	75	F	—	Nodes (peripheral), abdominal tumor	5	PR	ND	Nodes, abdomen	122+
8	37	M	—	Liver, nodes (peripheral), abdominal tumor	6	PR	57/32	Nodes, abdomen	183+
9	61	F	—	Liver	4	PR	35/31	Liver	92+
10	51	F	—	Liver, adrenals	8	NC	52/32	—	197
11	71	M	—	Liver	14	NC	28/15	—	365
12	50	M	—	Liver	10	NC	800/830	—	244+
13	47	M	—	Liver	5	PD	201/210	—	0
14	70	M	—	Liver, neoplastic ascites	3	PD	120/200	—	0
15	61	M	—	Liver, neoplastic ascites	2	PD	ND	—	0
16	45	M	—	Liver	3	PD	64/75	—	0
17	48	M	(a)	Liver	13	CR	19/<2.5	Liver	318+
18	37	M	(b)	Liver	5	PR	60/7.5	Liver	136+
19	56	M	(c)	Liver	6	PR	1205/292	Liver	152
20	49	M	(d)	Liver	8	NC	1030/92	—	228+
21	52	F	(b)	Liver	4	NC	22.2/19.3	—	90+
22	53	M	(e)	Liver	6	NC	550/200	—	153+
23	46	F	(e)	Liver	9	NC	18.8/17	—	305+
24	51	M	(f)	Liver	10	NC	23/35	—	244
25	41	M	(g)	Liver, nodes (peripheral)	3	PD	5/4	—	0
26	72	M	(g)	Bone, abdominal tumor	5	PD	14/16	—	0
27	60	M	(e)	Liver	3	PD	68/86	—	0
28	30	M	(g)	Liver, lung, abdominal tumor	3	PD	162/93	—	0
29	65	M	(e + h)	Liver	3	PD	ND	—	0
30	40	M	(g + h)	Liver	5	PD	100/100	—	0
				Patients with gastric adenocarcinoma					
1	69	F	—	Abdominal wall, stomach, liver	4	PR	ND	Liver, abdominal wall	90+
2	63	M	—	Liver, stomach	4	PR	70/15	Liver, stomach	90+
3	34	F	—	Liver, neoplastic ascites	2	PR	ND	Liver, ascites	60+
4	67	M	—	Nodes (mediastinal), stomach	3	NC	6.8/5	—	60+
5	71	M	—	Liver, abdominal tumor	4	PD	ND	—	0

*Previous treatment (only doses of 5-FU are given): (a) = 5-FU (400 mg/m^2/day × 5); (b) = doxorubicin + mitomycin + 5-FU (400 mg/m^2/day × 5); (c) = cyclophosphamide + mitomycin + 5-FU (400 mg/m^2/day × 4); (d) = 5-FU (400 mg/m^2/wk); (e) = cyclophosphamide + 5-FU (400 mg/m^2/day × 5); (f) = doxorubicin + cyclophosphamide + 5-FU (400 mg/m^2/day × 4); (g) = lomustine + 5-FU (400 mg/m^2/day × 4); and (h) = mitomycin + 5-FU (500 mg/m^2/wk).

†CR = complete response; PR = partial response; NC = no change; and PD = progressive disease; ND = not determined.

dures. Patient ages ranged from 30 to 75 years (mean ± SD, 53.2 ± 11). Before entering the trial, 14 patients had been subjected to an ineffective regimen of 5-FU given either as a single agent or combined with other drugs; the other 16 patients had not received previous chemotherapy (table 1).

Patients with gastric adenocarcinoma.—Six patients were entered in the study; one was excluded because neoplastic ascites was the sole indicator of disease. Five previously untreated patients with measurable disease were evaluated (table 1). Their ages ranged from 34 to 71 years (mean ± SD, 60.8 ± 15). The presence of metastases was proved histologically in all cases.

Treatment comprised 5-day courses followed by drug-free intervals of 21 days. FA was given at a dose of 200 mg/m²/day by iv bolus injection, and 5-FU was given immediately afterwards at a dose of 370 mg/m²/day by iv infusion over 15 minutes. In subsequent courses, the daily dose of 5-FU was raised to 400 mg/m² if toxicity was absent or mild at 370 mg/m²/day. The daily dose of 5-FU was reduced by 30 mg/m² in cases of granulocytopenia of < 1000 polymorphonuclear neutrophils (PMN)/mm³ complicated by fever, thrombocytopenia of < 50,000 platelets/mm³, and/or World Health Organization (WHO) grade 3 or 4 oral mucositis or diarrhea (9). Treatment courses were repeated until failure became evident or until progressive disease developed in previously responding or previously stable patients.

Before each course, each patient had a physical examination, peripheral blood cell counts including differential, liver function tests, and determinations of serum creatinine, serum electrolytes, glucose, and BUN. CEA was determined in 29 patients. Measurable disease was initially documented by physical examination and by anatomically oriented tests (chest roentgenogram, computerized tomographic [CT] scan, ultrasonography [USG], endoscopy, and/or radionuclide scan [RNS]) which were repeated during the follow-up period. Response was determined quantitatively by comparison of objectively measurable lesions and was categorized as CR, PR, no change, or progressive disease. CR was defined as the disappearance of all perceptible tumors, and PR was defined as a reduction in the sum of the products of the longest perpendicular diameters of all measurable lesions by at least 50% in the absence of any new site of malignancy. Patients in whom a 50% tumor regression could not be established and those in whom stable disease was recorded for at least 3 months were considered to have no change. Progressive disease was defined as the appearance of any new lesion and/or growth of existing lesions. Duration of response was determined from the start of therapy.

RESULTS

Patients With Colorectal Cancer

Of the 16 previously untreated patients, one achieved a CR and eight a PR. The complete responder had disappearance of pelvic and vaginal tumors as demonstrated by endoscopy and laparotomy. Of the partial responders, four patients with hepatic metastases as the sole indicator had tumor regression, which was measured in one patient by laparotomy, in two by both RNS and USG, and in one by USG. Another patient had reduction of pulmonary nodules and of liver metastases, as measured by CT scan, USG, and RNS, as well as disappearance of obstructive jaundice. One patient had regression of abdominal masses and of hepatic metastases, as measured by both RNS and USG. Finally, two patients had regression of metastatic nodes and abdominal masses. The response rate in this group of patients was 56%; responses lasted from 92 + to 258 + days (mean, 188).

Of the 14 patients previously resistant to 5-FU, one attained a CR and two a PR. The complete responder had disappearance of hepatic metastases, as evidenced by both RNS and CT scan. The two partial responders had regression of hepatic metastases, evidenced in one case by CT scan and in the other by RNS. The response rate in this group was 21%; responses lasted 136 +, 152, and 318 + days. The overall objective response rate for the 30 patients with colorectal adenocarcinoma was 40%.

TABLE 2.—Myelosuppression in 16 patients who had weekly blood cell counts during 2 mos for 2 consecutive courses at each dose level of 5-FU

Course	5-FU dose (mg/m²/day × 5)	Mean nadir (range)		Mean Day of nadir from Day 1 of treatment (range)	Mean time of recovery in days (range)
		PMN*	Platelets†		
1	370	29.9 (8–80)	296 (140–472)	13 (12–19)	8 (7–9)
2	400	19.2 (1–64)	238 (120–350)	14 (12–19)	8 (4–14)

* × 10^2 cells/mm^3.

† × 10^3 cells/mm^3.

Patients With Gastric Cancer

Of the five evaluable patients, three achieved a PR which lasted for 60+, 90+, and 90+ days. Tumor regression was very rapid and resulted in a dramatic reduction of tumor size. In one patient, a large lesion of the abdominal wall disappeared; another had regression of a gastric tumor as measured by endoscopy, and in another, neoplastic ascites disappeared. All three responding patients had regression of hepatic metastases as measured by USG.

Toxicity

Myelosuppression was studied in 16 patients who had blood cell counts weekly for 2 months during two consecutive courses at two different dose levels of 5-FU; the results (table 2) indicate that myelosuppression was moderate, with rapid recovery.

The overall toxicity is indicated in table 3. The 35 patients received a total of 207 courses of therapy, of which 198 were evaluated for toxic effects. The 5-FU doses were 370 mg/m^2/day × 5 (88 courses) and 400 mg/m^2/day × 5 (110 courses). The mean number of courses per patient was six (range, two to 14). Granulocytopenia of < 2000 PMN/mm^3 was recorded in 17 courses in 13 patients. Seven of these patients had been treated previously with chemotherapy or radiotherapy. Granulocytopenia of < 1000 PMN/mm^3 occurred in seven courses; six of these were complicated by fever, which in one case was due to *Staphylococcus aureus* septicemia. The six episodes of fever occurred at the higher dose of 5-FU. Five episodes of thrombocytopenia with $< 150{,}000$ platelets/mm^3 were recorded in four patients; three episodes of thrombocytopenia with $< 100{,}000$ platelets/mm^3 occurred in three pretreated patients. There were no hemorrhagic complications. No cumulative myelosuppression was detected in comparisons of PMN and platelet counts obtained at the end of the 21-day intervals in the 26 patients who received at least four courses of treatment (table 4).

Twenty-five episodes of oral mucositis were recorded in 17 patients and were severe (WHO grade 3 or 4) in five courses at the higher dose of 5-FU; the mucositis began on Days 6–10 and persisted for 7–10 days. Eleven episodes of diarrhea were noted in nine patients and were severe in two (WHO grade 3). Partial alopecia was seen in four patients. Moderate skin rash associated with conjunctivitis was recorded in two patients. Mild nausea was observed in most patients during the treatment courses. Neither renal nor neurologic toxicity was observed. There was no death due to toxicity. Toxic effects required 5-FU dose reductions in two courses at 370 mg/m^2/day (2%) and in 11 courses at 400 mg/m^2/day (10%).

TABLE 3.—Overall toxicity in 35 patients treated with 5-FU and high-dose FA

Toxic effect and degree	No. of patients experiencing toxic effects	No. of courses with episodes of toxicity, according to 5-FU dose	
		370 mg/m^2/day (88 courses)	400 mg/m^2/day (110 courses)
Granulocytopenia (× 10^2 PMN/mm^3)			
15–20	3	5	1
10–15	4	2	2
< 10	6	1	6
		8	9
Thrombocytopenia (× 10^3 platelets/mm^3)			
100–150	1	1	1
50–100	2	1	1
< 50	1	1	0
		3	2
Oral mucositis*			
1	6	7	4
2	6	4	5
3	4	0	4
4	1	0	1
		11	14
Diarrhea*			
1	0	0	0
2	7	4	5
3	2	1	1
4	0	0	0
		5	6
Fever	6†	0	6
Alopecia	4‡		
Skin rash	2		
Neurologic	0		
Renal	0		

*Graded according to WHO recommendations (9).

†In 5 patients, the cause of fever was not documented microbiologically; in 1 it was due to *Staphylococcus aureus* septicemia.

‡Partial.

DISCUSSION

The action of 5-FU on cell metabolism occurs through two pathways: one involves the incorporation of 5-FU into RNA, and the other, conversion of 5-FU into fluorodeoxyuridylate (FdUMP), which inhibits TS and results in the suppression of DNA synthesis by depletion of thymidine triphosphate (10,11). FdUMP binds tightly to TS in the presence of the cofactor N_5-N_{10} methylene tetrahydrofolate (CH_2FH_4); this interaction leads to the formation of a slowly dissociating covalent ternary complex (TS-CH_2FH_4-FdUMP). In the absence of CH_2FH_4, binding of FdUMP to TS is noncovalent and weak (4, 12).

In folate-depleted L1210 leukemia cells, both the amount of ternary complex formed and the cytotoxicity of 5-fluorodeoxyuridine are reduced (4). In mouse Sar-

TABLE 4.—Mean PMN and platelet counts obtained at end of 21-day treatment intervals in 26 patients who received at least 4 courses*

Courses	Mean PMN count ($\times 10^2/mm^3$)	Mean platelet count ($\times 10^3/mm^3$)
1	51.9	341
2	48.1	355
3	40.2	311
≥ 4	45	305

*Variance analysis shows no significant difference either for PMN or for platelet counts obtained at the end of each time period in the 26 patients.

coma 180 and in human carcinoma Hep 2 cells, the cytotoxic activity of 5-FU was potentiated threefold when an excess of FA was added to the medium (5). Both experiments (4,5) showed that the folate requirement for optimal cell growth was much lower than that required for maximal cytotoxicity of fluoropyrimidines. In Friend leukemia cells, the growth-inhibiting effect of 5-FU was also increased by addition of FA; this potentiation did not occur in FA transport-defective cells (6). Moreover, in xenografts of human colorectal carcinoma cell lines resistant to fluoropyrimidines, the maximum binding of FdUMP to TS requires addition of exogenous folates (7).

For the above reasons, we assumed that in some tumors, the amounts of naturally occurring intracellular folates may be insufficient to allow optimal cytotoxicity of 5-FU. It has also been reported that if low doses of FA (3 mg) are administered in combination with 5-FU and semustine in patients with advanced colorectal carcinoma, the survival rate is not improved (13). We therefore thought that high doses of FA should effectively increase the cellular content of reduced folates and that they might overcome membrane transport deficiency for these compounds (6,14).

In previously untreated patients with colorectal cancer, we obtained a response rate (CR + PR) of 56%; in previously 5-FU-resistant patients, the response rate (CR + PR) was 21%. These objective response data suggest that simultaneous administration of 5-FU and high doses of FA can enhance the antitumor activity of 5-FU and that it also renders some colorectal tumors, previously resistant to 5-FU, sensitive to this drug. For gastric adenocarcinoma, the results were also encouraging in the small number of patients studied and during the short follow-up period; PR were recorded for three of five previously untreated patients.

In the present trial, toxicity was within acceptable limits. Side effects, when present, were prevented in subsequent courses by a 30-mg/m^2 decrease in the daily dose of 5-FU.

The combination of 5-FU with simultaneous high doses of FA should be tested in all tumors in which 5-FU has shown some therapeutic effectiveness. Biochemical and pharmacokinetic studies will be necessary for optimization of the doses and schedules of FA combined with 5-FU and other cytostatic fluoropyrimidines.

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Dianhydrogalactitol and Cisplatin in Combination for Advanced Cancer of the Uterine Cervix[1,2]

Steven E. Vogl,[3,*] **Vicki Seltzer,**[3] **Fernando Camacho,**[4] **and Antonio Calanog**[3,5]

Eighteen evaluable patients with squamous cancer of the uterine cervix recurrent after radiation therapy or with distant metastases were treated with dianhydrogalactitol (150 mg/m^2) and cisplatin (DDP) (50 mg/m^2), both given iv every 3 weeks. Only 39% of the patients responded, a result no better than that previously reported for DDP alone and only one-half as good as that previously reported by our group for a combination of this drug with mitomycin, vincristine, and bleomycin. Responses lasted a median of only 5 months. Hematologic toxicity was life-threatening in 22% of the patients and was severe in all but 11% of the remaining patients. We conclude that the addition of substantial and toxic doses of dianhydrogalactitol to DDP failed to substantially enhance the rate, quality, or duration of response compared to DDP alone. [Cancer Treat Rep 66:1809–1812, 1982]

Chemotherapy for advanced cancer of the uterine cervix is generally unsatisfactory. Cisplatin (DDP) is the single most active agent (1). In a study by the Gynecologic Oncology Group (GOG) using 50 mg/m^2 of DDP every 3 weeks, 11 of 22 patients without prior chemotherapy responded, while only two of 12 exposed to prior chemotherapeutic agents had objective responses (1). The median duration of response, measured from the start of remission to evidence of disease progression, was 6 months. Complete remission was noted in only three (14%) of the 22 patients given DDP as initial chemotherapy. Other agents, particularly doxorubicin and cyclophosphamide, have little activity alone or in combination (2).

In earlier pilot trials, we added DDP to combination programs of established efficacy: methotrexate plus bleomycin (3) and mitomycin plus vincristine plus bleomycin. Responses were frequent but short (4,5), and toxicity was severe with the former regimen.

In 1980, Blom et al (6) reported for the GOG that dianhydrogalactitol (DAG), an alkylating agent formulated for iv administration (7), was active against squamous cancers of the uterine cervix (6). In the GOG study, seven of 29 patients without prior chemotherapy (24%) responded to this drug; the responses included two complete remissions, one of which lasted > 22 months. The median duration of response was 4 months among all seven responding patients. No responses were noted among six other patients with prior chemotherapy. The dose of DAG in this trial was 60 mg/m^2 iv every 7 days.

The toxicity of DAG is predominantly hematologic, with infrequent and inconsistent reports of mild nausea and vomiting and occasional stomatitis. No end-organ toxicity other than myelosuppression has been reported (6,8–12). This makes DAG well-suited for combination with DDP, since the latter is only mildly myelosuppressive but can be nephrotoxic and produces moderately severe nausea and vomiting in most patients treated (1). The aims of the present study were to develop a safe combination chemotherapy schedule combining DAG and DDP and to make a preliminary estimate of the

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[1] Received Mar 29, 1982; revised June 9, 1982; accepted July 12, 1982.

[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported in part by Public Health Service grants CA-13330 and CA-14958 (National Cancer Institute) and RR-00050 (Division of Research Resources) from the National Institutes of Health, Department of Health and Human Services; by the Chemotherapy Foundation; and by the New York City Division of the American Cancer Society.

[3] Division of Medical Oncology, Department of Medicine (*S. E. Vogl*) and Division of Gynecologic Oncology, Department of Gynecology (*V. Seltzer* and *A. Calanog*), Albert Einstein College of Medicine of Yeshiva University, Bronx, NY.

[4] Department of Oncology, Montefiore Hospital and Medical Center, Bronx, NY.

[5] We thank Drs. Josh C. Tunca and Thomas E. Davis of the University of Wisconsin (CA-21076) for their assistance in the conduct of this trial, and Dr. Cyrus Mehta, Sidney Farber Cancer Institute, for statistical support.

[*] *Reprint requests to:* Steven E. Vogl, MD, Division of Medical Oncology, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461.

activity of the combination to determine if further investigations are indicated.

The choice of DAG dose was based on phase I trials (9) and on a phase II study in non-small cell lung cancer (11), where a dose of 150 mg/m^2 divided over 5 days every 4 weeks produced moderately severe hematologic toxicity with some frequency. In the treatment of glioblastoma multiforme, a single bolus dose of 150 mg/m^2 every 3 weeks had produced acceptable toxicity with the advantage of a more convenient schedule than daily administration for 5 days (12). A single dose of DAG every 3 weeks was chosen for the present combination trial, to be given with 50 mg/m^2 of DDP (the effective dose in the GOG study), to allow for an accurate definition of nadir blood cell counts in each cycle as a basis for later dose modification. This was believed to be safer than the administration of small weekly doses, since the weekly schedule would allow the administration of further DAG at a time when toxic doses had already been administered but hematologic toxicity was not yet fully apparent.

PATIENTS AND METHODS

Eligibility was restricted to women with squamous or adenosquamous cancer of the uterine cervix with disease that could be measured in two diameters (or at least evaluated for response to chemotherapy), serum creatinine $\leq$ 1.5 mg/dl, wbc count $\geq$ 4000/µl, platelet count $\geq$ 100,000/µl, no prior chemotherapy with any protocol drugs, and tumor beyond hope of cure by surgical excision or radiation therapy. The patient was required to give informed consent prior to entry in the study.

Protocol drug administration included 50 mg/m^2 of DDP and 150 mg/m^2 of DAG iv every 3 weeks. DDP was administered 30 minutes after 40 mg of iv furosemide and the beginning of a 2-hour infusion of 2 L of 5% dextrose in 0.45% NaCl containing 10 mEq of KCl/L. Mannitol (12.5 g) was given just prior to the DDP. This regimen of DDP administration has proved safe and effective for outpatient use (13). DAG was administered over 1 hour in 1 L of fluid, usually the second liter of iv 5% dextrose in 0.45% saline stipulated in the protocol for DDP administration.

Doses were reduced to 67% for wbc counts of 3000–4000/µl or platelet counts of 75,000–100,000/µl. Chemotherapy was withheld on a week-by-week basis if either blood cell count was below the stipulated level. Blood cell counts were routinely obtained on the days of planned chemotherapy and 2 weeks thereafter. Any wbc count $<$ 1500/µl, granulocyte count $<$ 750/µl, or platelet count $<$ 40,000/µl led to a permanent reduction of DAG dose to 100 mg/m^2, which could be further reduced if blood cell counts on the day of planned chemotherapy were depressed.

Complete remission was defined as the complete disappearance of all signs and symptoms of disease. Partial response was defined as a $\geq$ 50% decrease in the sum of the products of perpendicular cross-sectional diameters of measurable lesions. Disease progression was defined as an increase of $\geq$ 25% in the sum of the products of perpendicular diameters of measurable or evaluable lesions, the appearance of new lesions, a $>$ 10% weight loss, or a decrease in performance status $>$ 1 point on the Eastern Cooperative Oncology Group (ECOG) scale (0 = fully functional; 1 = ambulatory with symptoms; 2 = bedridden less than one-half of the day; 3 = bedridden more than one-half of the day; and 4 = fully bedridden).

Treatment was continued until disease progression was noted. A minimum of two cycles of chemotherapy were planned for each patient.

Twenty-three patients were entered in the trial between November 1979 and March 1981. Five patients have been excluded from the present analysis. One patient has no follow-up available, and two started chemotherapy with markedly reduced doses of one or both agents and were never given full doses. Neither of these patients responded, but one did have life-threatening hematologic toxic effects. Since response might have

TABLE 1.—Patient characteristics

	All patients (18)	Responders (7)	Nonresponders (11)
Prior chemotherapy	6%	0%	9%
Performance status 0 or 1	78%	71%	82%
Weight loss > 5% in 6 mos	39%	45%	29%
Age in yrs			
Median	52	51	53
Range	40–65	46–65	40–65
Mos from diagnosis to entry			
Median	11.5	11	12
Range	0–121	0–118	6–121

TABLE 2.—Response according to site of involvement

Site	No. of patients— Responding	With involvement
Pelvis and other sites	1	6
Pelvis only	2	5
Abdominal wall	2	3
Lung	2	3
Para-aortic nodes (by computerized tomography)	1	6

occurred at full doses in one or both of these patients, they are excluded from further analysis. The other two patients who are excluded from analysis died 2 weeks after the first dose of chemotherapy, presumably of disease, but without further follow-up information available. The inclusion of any or all of these five patients in the present analysis would not alter the conclusions reached. Their exclusion, however, allows a more clear-cut definition of toxicity and efficacy.

Some of the patient characteristics are summarized in table 1. Only one patient was bedridden more than one-half the day, and only five patients had lost > 10% of their body weight in the previous 6 months. All patients had squamous cancer of the uterine cervix with a single exception, an adenosquamous cancer in a patient who did achieve partial remission. The pathologist at the time of diagnosis called tumor well-differentiated in three patients, moderately differentiated in six, and poorly differentiated in five. Differentiation was unknown in four patients. The interval from diagnosis to entry was between 0 and 13 months in 11 patients, 27 and 49 months in four patients, and 116 and 121 months in three patients.

One patient who had lung metastases at presentation had received no prior anticancer therapy, 12 had had prior radiation therapy alone, four had had prior major surgical procedures as well as irradiation, and one had had radiation therapy and single small doses of methotrexate and bleomycin.

Sites of involvement followed for response are given in table 2. More than one site was followed in six patients.

RESULTS

Among the 23 patients entered, 18 of whom were considered fully evaluable for response and toxicity, two achieved complete remission for 5 ½ and 21+ months, and five achieved partial remissions for 2+, 3, 4, 5, and 6 months.

One nonresponding patient received only one dose of chemotherapy before she refused further therapy (her abdominal computerized tomogram was unchanged at this point). The other ten nonresponding patients received two doses (four patients), three doses (one), four doses (one), five doses (three), and nine doses (one) of DAG-DDP before disease progression was apparent. Table 1 fails to reveal patient characteristics predictive of response to DAG-DDP. Four of 11 patients with intervals from diagnosis to start of chemotherapy between 0 and 13 months responded, as did one of four with intervals of 27–49 months and two of three with intervals of 116-121 months. The one patient without prior radiation therapy responded, as did two of four with prior surgery plus radiation therapy. As shown in table 2, responses were seen at least once in each site that was evaluated for response.

Median survival is 9 months among the 18 evaluable patients. Four of seven responders have died. The median survival of the responders is projected at 15 months and will not be < 10.5 months. Two responders are alive (one with obvious disease) at 21 and 24 months from entry. Median survival of the 11 nonresponders, eight of whom have already died, is 8 months, with the longest survival being 12 months from entry. Responders did not live significantly longer than nonresponders (log-rank test).

Toxicity

As anticipated from the dose of DAG chosen, the major toxicity was hematologic. Fourteen patients (78%) required packed rbc transfusions. Three (17%) had platelet counts < 25,000/µl at some point, with major hemorrhage from vagina and rectum in one patient each. Four more patients (22%) had nadir platelet counts of 25,000–49,999/µl, and five others (28%), 50,000–74,999/µl. Nadir wbc counts < 1000/µl were seen in two patients (11%); 1000–1900/µl, in seven (39%); and 2000–2999/µl, in seven (39%). Two patients had increases of the serum creatinine to > 1.5 mg/dl; one patient had a peak level of 1.7 mg/dl, and another had a peak level of 1.6 mg/dl, which first rose only several weeks after DDP administration but returned to the pretreatment level of 1.3 mg/dl promptly on rehydration. Thus, there was only one instance of mild azotemia from DDP. One patient had repeated anaphylactic shock from DDP in spite of steroid and antihistamine pretreatment, which led to cessation of therapy 16 months after having achieved a complete remission. One patient developed a perforated duodenal ulcer while in remission 2 days after her second dose of chemotherapy, and survived surgical plication. The relation of this event to the chemotherapy is uncertain.

DISCUSSION

The results observed with a combination of DAG and DDP are not substantially better than those previously reported for DDP alone, although the number of patients given the combination was small. Among patients without prior chemotherapy, moderate doses of DDP alone yielded responses in 50% of patients treated, with a median response duration of 6 months and a median survival of 9 months among responding patients (1). While the response rate has been only 25% in a subsequent GOG trial of different doses of DDP in cervical cancer (14), the response rate of 39% observed in the present trial remains in the range of that seen with DDP alone.

The very high degree of hematologic toxicity observed with DAG-DDP argues that the DAG dose was sufficiently high, since this dose of DDP is only mildly myelosuppressive (1). Perhaps more activity would have been observed if DAG had been administered weekly, as it had been in the original positive phase II trial (6). Some schedule specificity has been observed for DAG in animal systems, but none has been demonstrated in man (8). In tissue culture, DAG cytotoxicity is independent of the phase of the cell cycle (7). Moderate antitumor efficacy (15% responses) in human squamous cancer of the lung has been demonstrated for DAG at a dose of 30 mg/m^2 iv daily for 5 days every 4–5 weeks.

The results of the present trial are particularly disappointing when compared to the previous results observed with DDP combined with mitomycin, vincristine, and bleomycin (BOMP). Responses with both regimens were short (median of 4 months for BOMP and 5 months for DAG-DDP), but the response rate was twice as high for BOMP as for DAG-DDP (78% vs 39%) (5). The 18 evaluable patients in the DAG-DDP study were quite similar in clinical characteristics to the 13 evaluable patients in the BOMP study, so that the apparent superiority of BOMP is unlikely to relate to patient selection.

We conclude that the addition of DAG to DDP in the chemotherapy for advanced cancer of the uterine cervix did not substantially improve response rate or response duration, while it did produce substantial hematologic toxicity. Major resources should not be expended conducting phase III comparative studies of DAG-containing combination chemotherapy programs in cervical cancer unless other pilot studies provide a great deal more favorable evidence of its activity in combination than was found in the present trial.

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Phase I–II Study of *m*-AMSA Administered as a Continuous Infusion[1]

Kenneth C. Micetich,* Leonard A. Zwelling, Paul Gormley, and Robert C. Young[2]

In vitro studies of 4′(9-acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA) with L1210 leukemia cells have demonstrated that a given level of DNA breakage and intracellular *m*-AMSA concentration depends upon the presence of a continuous concentration of extracellular drug and that cytotoxicity increases with an increasing duration of drug exposure. Since previous studies had shown that free *m*-AMSA has a half-life of 6–8 hours, a trial of *m*-AMSA given as a continuous infusion was undertaken in patients with solid tumors and lymphomas. Patients were treated with 30 mg/m^2/day × 3, 30 mg/m^2/day × 4, or 40 mg/m^2/day × 3. Myelosuppression was dose-limiting and occurred at about Day 13 after the start of the infusion. Recovery was noted by Day 21. There was no evidence of cumulative hematologic toxicity. The maximally tolerated dose was 30 mg/m^2/day × 3 in our patient population. Mild phlebitis occurred in all patients. There were no instances of gastrointestinal, hepatic, renal, cardiac, or neurologic toxic reactions. Evidence of some antitumor effect was seen in three patients. We conclude that it is possible to give *m*-AMSA as a continuous infusion and that studies should be undertaken in patients with acute myelogenous leukemias to compare the efficacy of *m*-AMSA infusion with conventional bolus administration as a means to enhance the duration of response.

[Cancer Treat Rep 66:1813–1817, 1982]

4′-(9-Acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA) has been shown to be a promising agent in the treatment of anthracycline-resistant myelogenous leukemias (1,2). In the treatment of solid tumors, the commonly employed means of drug administration is either a single large bolus iv (120 mg/m^2 every 3–4 weeks) or smaller daily boluses iv (30–40 mg/m^2/day for 3 days every 3–4 weeks) (3,4). The dose-limiting toxic effect of these two schedules has been myelosuppression, occurring about 10–14 days after drug administration, with recovery by Day 21. Much higher doses have been used in the treatment of acute leukemias, and with higher doses, mucositis and gastrointestinal toxicity have been significant problems (1,5). Cardiac and neurologic toxic effects have been reported, although the exact incidence of either of these two complications is unknown (6,7).

In man, *m*-AMSA exists in the serum as both free and bound parent drug and metabolites. The principal route of excretion appears to be the biliary system (8,9). Previous pharmacokinetic studies have shown that free *m*-AMSA is cleared from the serum with an initial half-life of 11–15 minutes and a slower terminal half-life of 6–8 hours (9,10). By 24 hours after a single iv bolus, the free drug has decreased by a factor of 100 (11). What is not known, however, is the optimal concentration of *m*-AMSA and duration of exposure for the drug to be cytotoxic to human malignant cells.

Recent work has shown that DNA single-strand breaks produced by *m*-AMSA in L1210 cells disappeared within 20 minutes following removal of the drug (12). Second, drug egress from the cells in transport studies was very rapid following removal of the drug from the extracellular medium (12). Finally, soft agar colony survival studies using L1210 cells have suggested a schedule dependency; ie, a low concentration of drug for long exposure times was more cytotoxic than a much higher concentration of drug for short exposure times (L. A. Zwelling, D. Kerrigan, and S. Michaels, manuscript submitted).

Thus, since a given level of DNA breakage and intracellular *m*-AMSA concentration depended upon the

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[1] Received Jan 18, 1982; revised June 23, 1982; accepted July 21, 1982.

[2] Laboratory of Molecular Pharmacology (*K. C. Micetich* and *L. A. Zwelling*), Laboratory of Chemical Pharmacology (*P. Gormley*), and Medicine Branch, Clinical Oncology Program (*R. C. Young*), Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Kenneth C. Micetich, MD, Loyola University Medical Center, 2160 S First Ave, Maywood, IL 60153.

presence of a continuous concentration of extracellular *m*-AMSA, since cytotoxicity increased with increasing duration of drug exposure, and since the half-life of the free drug is short, we proposed a trial of *m*-AMSA administered as a continuous infusion.

MATERIALS AND METHODS

Clinical Study

Twenty-two patients were entered in the study over a 6-month period. Patient characteristics are shown in table 1. Fourteen females and eight males received 31 courses of the drug. All courses were evaluable for toxicity, and 20 of the 22 patients were evaluable for response. The median age was 50 years and the median performance status was 70%. Lymphoma, breast cancer, and ovarian cancer were the most frequent diagnoses in the study population. All patients except two had been pretreated with drug regimens containing at least four myelosuppressive agents, and 14 of the 22 patients had received doxorubicin either as a single agent or in combination prior to receiving *m*-AMSA. Nine of the 22 patients had also been previously treated with radiotherapy.

TABLE 1.—Characteristics of patients treated with *m*-AMSA

Characteristic	No. of patients
Total in study	22
Females	14
Males	8
Median age in yrs (range): 50 (23–67)	
Median Karnofsky performance status: 70%	
Diagnosis	
Lymphoma	7
Breast cancer	5
Ovarian cancer	5
Sarcoma	2
Melanoma	2
Testicular cancer	1
Previous treatment	
Radiotherapy + chemotherapy	9
Chemotherapy alone	13

Prior to entry in the study, each patient had a history and physical examination, ECG, cbc with differential and platelet counts, routine automated chemistries, and appropriate baseline radionuclide scans and X-rays. To be eligible for study, the patients must have had evaluable disease, a life expectancy of at least 1 month, normal renal and hepatic function tests, a wbc count $\geqslant 3500/\mu l$, and a platelet count $\geqslant 100{,}000/\mu l$. Exceptions were made in any of the patients with abnormal pretreatment hemograms or liver function tests if the abnormalities were thought to be due to malignancy. Informed consent was obtained in writing from all patients.

All treatments were given on an inpatient basis. Initial patients entered in the study were treated with 30 mg/m^2/day for 3 days. If no toxic reaction was seen, subsequent patients were treated with 30 mg/m^2/day for 4 days. The intent was to increase the infusion by 24 hours with every three patients until the dose-limiting toxicity was reached. Further patients would then be given 40 mg/m^2/day for 3 days, with escalations as above until the dose-limiting toxicity was reached. Thus, it would be possible to determine a maximally tolerated dose and infusion duration for patients treated with either 30 or 40 mg/m^2/day.

Solutions of *m*-AMSA were prepared in the pharmacy. The appropriate amount of *m*-AMSA in a vehicle of L-lactic acid and *N*, *N'*-dimethylacetamide was added to 1000 ml of 5% dextrose in water (D5W), to which 1 mEq of sodium bicarbonate had been added. The resultant solution was infused via a peripheral vein utilizing an IVAC pump (IVAC Corp, San Diego). Each liter of solution was administered over a 12-hour period. One patient with only one arm and two other patients who had no peripheral venous access site had the drug administered via a central vein.

Four patients who had either previously received 550 mg/m^2 of doxorubicin or who had cardiac symptoms underwent continuous ECG monitoring during the infusion of *m*-AMSA.

After the infusion, patients were discharged and interim blood cell counts and liver function tests were obtained twice weekly. Re-treatment was expected every 21–28 days or when peripheral blood cell counts had recovered. After two cycles of drug treatment, patients were evaluated as to disease status. If disease progression had occurred, treatment was discontinued. Patients were evaluated according to standard response criteria defined as follows: *complete remission*—the disappearance of all evaluable disease; *partial remission*—a $> 50\%$ decrease in the product of the two longest perpendicular diameters, lasting 4 weeks; *disease stabilization*—a $< 50\%$ decrease in the product of the two longest perpendicular diameters, lasting 4 weeks; and *disease progression*—the appearance of new lesions or a $> 25\%$ increase in the product of the two longest perpendicular diameters. To be evaluable for response, patients had to have received at least one course of drug and been examined at the National Cancer Institute to determine disease status prior to death.

Pharmacokinetics

Blood samples were obtained by venipuncture at 12–24-hour intervals following the start of the infusion. A 2-ml aliquot of serum was added to 10 ml of ethyl acetate and the mixture was shaken vigorously. Under these conditions nearly 100% of free *m*-AMSA will extract into the ethyl acetate. The ethyl acetate (5 ml) was separated from the aqueous phase and stored at −20°C until further analysis (10). Preparation for high-performance liquid chromatography analysis consisted of evaporating the 5 ml of ethyl acetate to dryness and resuspending the residue in 2 ml of 0.01 M phosphate buffer (pH 8). The sample was adjusted to pH 2 by the addition of 0.5 ml of HCl (0.5 N). The solution was extracted with 7 ml of *n*-hexane and the hexane was then discarded. A solution of saturated sodium borate (3 ml) was added to the remaining aqueous phase, and the solution was extracted twice with 5 ml of ethyl acetate. The ethyl acetate fractions were pooled and evaporated to dryness. The residue was dissolved in 200 μl of a solution of methanol:water (9:1). A 100-μl sample was injected onto an Altex high-performance liquid chromatography system, using a 10-μl Spherisorb ODS 4.2 × 25-cm column. The mobile phase was methanol:0.004 M sodium heptanesulphonate (4:1) pumped at 2 ml/minute, and the column was monitored at 254 nm with a Gilson model 111 LC detector (L. Malspeis, unpublished method).

RESULTS

Pharmacokinetics

Free *m*-AMSA plateaued in the serum of patients by 24 hours and remained constant until the end of the infusion at 72 or 96 hours. The mean steady-state concentration of free *m*-AMSA in eight patients receiving 30 mg/m^2/day for 3 or 4 days was 0.15 ± 0.097 μM. Two patients who received 40 mg/m^2/day for 3 days had steady-state *m*-AMSA values of 0.11 and 0.25 μM, respectively.

Toxicity

The toxic reactions observed were mainly myelosuppression and phlebitis.

Myelosuppression

Blood cell count suppression as a function of drug dose is shown in table 2. At a dose of 30 mg/m^2/day for 3 days, 12 patients received 18 evaluable cycles of the drug: seven received one cycle, four received two cycles, and one received three cycles. The median time to retreatment was 34 days from the start of the *m*-AMSA infusion.

Eight drug cycles resulted in nadir wbc counts < 2000/μl and two in nadir wbc counts < 1000/μl. The median nadir wbc count was 2100/μl, occurring on about Day 13 with recovery by Day 20. The two patients who had nadir wbc counts < 1000/μl had breast cancer, a poor performance status, and a history of extensive irradiation. Two patients had febrile episodes during treatment and both recovered with broad-spectrum antibiotic administration.

Eight cycles produced a nadir platelet count < 50,000/μl. The median nadir platelet count was 36,000/μl, occurring on Day 15 with recovery by Day 21. One patient had a nadir platelet count < 20,000/μl but this was associated with advancing infiltrative marrow disease. There were no hemorrhagic episodes and no deaths attributable to drug toxicity in this group. In the five patients who received more than one cycle of drug, there was no evidence of a cumulative hematologic toxicity.

Seven patients received ten evaluable cycles of drug at a dose of 30 mg/m^2/day for 4 days: five patients re-

TABLE 2.—Hematologic toxicity of *m*-AMSA administered as a continuous infusion

	30 mg/m^2/day × 3	30 mg/m^2/day × 4	40 mg/m^2/day × 3
No. of patients	12	7	3
No. of cycles	18	10	3
No. of cycles with wbc count nadir < 2000/μl	8	6	3
No. of cycles with wbc count nadir < 1000/μl	2	5	3
Median wbc count nadir (range)/μl	2100 (500–7600)	1600 (600–4000)	200 (100–900)
Median day of wbc count nadir (range)*	13 (8–18)	15 (9–16)	13 (12–13)
No. of cycles with platelet count nadir < 100,000/μl	8	7	3
Median platelet count nadir (range)/μl	36,000 (11,000–525,000)	38,000 (3000–212,000)	109,000;49,000;7000 (individual values listed)
No. of febrile episodes	2	2 (1 septic death)	2 (1 septic death)

*All days are counted from start of *m*-AMSA infusion.

ceived one cycle, one received two cycles, and one received three cycles. Six cycles produced a nadir wbc count < 2000/μl, with five producing a nadir wbc count < 1000/μl. The median nadir wbc count was 1600/μl, occurring at about Day 15 with recovery by Day 22. The median nadir platelet count was 38,000/μl, occurring about Day 17. In this treatment group there were two febrile episodes, with one death due to sepsis. One patient required platelet transfusions for counts < 20,000/μl. There was no evidence of cumulative hematologic toxicity in the two patients who received multiple courses of the drug.

Only three patients were treated at a dose of 40 mg/m^2/day for 3 days and all three had severe myelosuppression (table 2). There were two febrile episodes in this group, with one death due to *Listeria* meningitis. Two of the three patients had breast cancer and a poor performance status and had received extensive treatment prior to receiving *m*-AMSA; one of these two had received lumbosacral irradiation concomitantly with *m*-AMSA treatment.

Phlebitis

All patients experienced phlebitis at the injection site. Initially, the daily dose of drug was administered in a total volume of 1000 ml of D5W over 24 hours. Under these conditions, the phlebitis was severe, causing significant discomfort by about 6 hours and usually necessitating a change in the iv site by 12 hours. Administering each day's dose of drug in a total volume of 2000 ml of D5W, with 1 mEq of sodium bicarbonate/L of solution, significantly lessened the phlebitis. Under these conditions of administration, phlebitis occurred by 24 hours and the iv site had to be changed every 24–36 hours. The phlebitis was characterized as tenderness and redness without warmth at the injection site and usually extended several inches beyond the iv site. The discomfort was tolerable; no infusion had to be stopped and no patient refused further therapy because of pain. The soreness in the arm resolved in about 3 days. Three drug extravasations occurred. There was no necrosis and only minor soreness for several days. Two of the patients were receiving corticosteroids for central nervous system metastases and both of these patients had minimal problems with phlebitis.

Other Toxicity

No patients had nausea or vomiting during the infusion. One patient developed mild mucositis on Day 7.

One patient with known liver involvement from embryonal cell carcinoma of the testes developed a transient rise of SGOT and SGPT, which peaked at Day 17 and became normal by Day 34. Seven patients developed elevations of alkaline phosphatase while receiving therapy, but all seven had known metastatic disease in the liver and the majority of them had evidence of disease progression in the liver as the alkaline phosphatase level rose.

None of the patients had ECG changes before, during, or after the *m*-AMSA infusion. Three of four patients who underwent constant ECG monitoring during the infusion showed no evidence of arrhythmia. The fourth patient, who had a history of Wolff-Parkinson-White syndrome and was receiving therapy with quinidine sulfate, had one episode of supraventricular tachycardia while receiving *m*-AMSA, but this terminated spontaneously. He had had occasional attacks of palpitations prior to receiving *m*-AMSA, however, despite being on antiarrhythmic therapy.

Responses

Evidence of some antitumor effect was seen in three patients. One patient with nodular poorly differentiated lymphoma had resolution of generalized lymphadenopathy by Day 4 of the infusion (30 mg/m^2/day for 4 days). This patient, who had received total-body irradiation 7 years previously, was unable to be re-treated because of persistent severe thrombocytopenia. The lymphadenopathy subsequently returned by Day 21.

The other two effects were seen in patients with ovarian cancer. One patient had a large pelvic mass with hydronephrosis and while receiving *m*-AMSA had a < 50% shrinkage of the mass, with improvement of the hydronephrosis lasting > 24 weeks. The other patient with ovarian cancer had disease stabilization for 16 weeks.

DISCUSSION

Two questions need to be answered when the schedule of drug administration is changed: (*a*) is the toxicity schedule-dependent and is the pattern of toxicity altered? and (*b*) is it efficacious to give the drug in this fashion?

We have demonstrated that the administration of *m*-AMSA as a continuous infusion is feasible. When administered via a peripheral vein, a mild to moderate phlebitis ensued which resolved within 3 days and was reasonably well-tolerated. Furthermore, except for the phlebitis, the pattern of toxicity was not altered by administering *m*-AMSA as a continuous infusion. Hematologic toxicity was dose-limiting and reversible. Thrombocytopenia was seen frequently. Published studies using *m*-AMSA as a single iv daily bolus have noted only mild thrombocytopenia when the platelets were affected (3,10,13). Thus, it is possible that the thrombocytopenia seen in our study might represent a schedule-dependent toxicity. However, three recent studies in patients with lymphoma, prostate cancer, and small cell lung cancer have noted a higher incidence of thrombocytopenia than that reported in other studies (14–16). These three studies suggest that the degree of thrombocytopenia in our data is not a reflection of schedule-dependent toxicity but rather reflects the pretreatment and poor bone marrow reserve of our patient population.

The maximally tolerated dose of *m*-AMSA in this study was 30 mg/m^2/day for 3 days. This dose produced wbc count nadirs $< 2000/\mu l$ and platelet count nadirs $< 50{,}000/\mu l$ in approximately 50% of the patients. We recommend this as the initial starting dose in adult patients with solid tumors who have poor performance status or poor bone marrow reserve or are receiving concomitant radiotherapy to large marrow-containing areas. Because of the extensive prior treatment received by our patients, our study cannot resolve the issue of the maximally tolerated dose of *m*-AMSA in patients who have good bone marrow reserve.

While we have demonstrated the feasibility of giving *m*-AMSA as a continuous infusion, the question of efficacy cannot yet be answered. Efficacy is the sum total of response rate balanced against overall toxicity. To have increased efficacy, a change in the drug schedule should demonstrate either (*a*) an increased response rate with no change or a decrease in toxicity or (*b*) no change in the response rate with a decrease in toxicity when compared to other schedules.

Recent studies have shown that when *m*-AMSA is given as a bolus to patients with solid tumors, the reponse rate is virtually zero. In fact, the clinical spectrum of activity of *m*-AMSA as a single agent is remarkably narrow, being relegated to treatment of the lymphomas and the myelogenous leukemias. We feel that in order to test efficacy, the schedule change we have studied for *m*-AMSA needs to be studied systematically in the myelogenous leukemias and the lymphomas, both tumors with known significant response rates to *m*-AMSA (1,2,5,14).

In the myelogenous leukemias, large bolus doses are used and have been associated with significant gastrointestinal toxicity (5). Continuous infusion might avoid the gastrointestinal toxicity. Our data demonstrated that steady-state values of free *m*-AMSA are reached at the end of the first day of infusion. More importantly, peak or steady-state levels achieved by continuous infusion are 1%–2% of those peak serum levels attained with bolus administration of the drug (10). Recently, the cardiac toxicity of doxorubicin has been found to be reduced by administering the anthracycline as a continuous infusion (17,18). Thus, if acute cardiac toxicity of *m*-AMSA is related to high peak serum concentrations of drug, then continuous infusion of AMSA might avoid this dramatic and sometimes lethal side effect. Finally, if concentration-times-time effects are important in killing human leukemia cells and if cytotoxicity of *m*-AMSA is schedule-dependent in man, then the continuous infusion of *m*-AMSA might improve the response rate and duration of responses in the leukemic population.

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Pharmacokinetic Studies of 4′-Epi-doxorubicin in Cancer Patients With Normal and Impaired Renal Function and With Hepatic Metastases[1]

C. M. Camaggi,[2] E. Strocchi,[3] V. Tamassia,[4] A. Martoni,[3] M. Giovannini,[3] G. Iafelice,[3] N. Canova,[3] D. Marraro,[3] A. Martini,[4] and F. Pannuti[3,]*

The plasma pharmacokinetics of 4′-epi-doxorubicin (4′-epiDX), a new anthracycline derivative, was investigated in 22 cancer patients after single iv bolus administration. Eleven patients had normal kidney and liver functions, five had impaired renal function, and six had liver metastasis. Plasma levels (up to 96 hours) of the unaltered drug and of one metabolite (4′-epi-doxorubicinol) were specifically determined by a highly sensitive high-pressure liquid chromatography method with fluorescence detection (in 19 patients after the first course and in five patients after the fourth course). A triexponential decrease in the plasma levels of the unaltered drug, with a long terminal plasma half-life, was observed in all patients. The metabolite levels were always lower than those of the parent compound. The main pharmacokinetic parameters of 4′-epiDX in patients with normal kidney and liver functions were (mean ± SE): half-life = 40 ± 6 hrs, plasma clearance = 0.882 ± 0.075 L/min, and volume of distribution = 46.1 ± 7.5 L/kg, suggesting that the extensive extravascular distribution is the main determinant of the slow elimination of the drug. No significant differences between normal and impaired renal function were found with respect to the pharmacokinetics of 4′-epiDX. In patients with liver metastasis, changes in the disposition of the drug were observed, which involved the area under the curve and plasma clearance. The observations suggest that in cancer patients with liver metastasis, dose modification of 4′-epiDX is required. [Cancer Treat Rep 66:1819–1824, 1982]

4′-Epi-doxorubicin (4′-epiDX) is a new anthracycline derivative closely related to doxorubicin (DX), with a broad spectrum of activity against animal tumors (1,2). Preclinical studies indicate less systemic and cardiac toxicity than that of DX at similar equipotent therapeutic doses (2).

4′-EpiDX is currently under extensive phase II clinical investigation. The results obtained so far suggest that the drug is as effective as DX against a broad spectrum of human neoplasms and better tolerated in terms of conventional toxicity (3–6). Specific studies of chronic cardiac toxicity are in progress. Experimental studies in animals (2,7), as well as a preliminary crossover comparative pharmacokinetic study in a group of cancer patients after single iv administration of 60–75 mg/m^2 of DX and 4′-epiDX (8), show a more rapid disappearance of 4′-epiDX in comparison with DX.

At present, no definite data are available as to the fate of this drug after administration in man, with particular reference to the metabolic pathway and routes of excretion. However, preliminary data (unpublished observations) indicate that urinary excretion of 4′-epiDX, as well as one of its metabolites, the 13-dihydro derivative 4′-epi-doxorubicinol (4′-epiDXol), accounts for only about 10% of the administered dose, while very high concentrations of both compounds are found in bile. It appears, therefore, that the fate of 4′-epiDX is qualitatively similar to that of DX.

The aim of the present study was to investigate some clinical pharmacokinetic aspects of 4′-epiDX with particular reference to interpatient variation in drug clearance and the possible pharmacokinetic consequences of renal impairment and liver metastasis.

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[1] Received Jan 4, 1982; revised June 3, 1982; accepted June 29, 1982.

[2] Istituto di Chimica Organica, Università degli Studi, Bologna, Italy.

[3] Divisione di Oncologia, Ospedale M. Malpighi, Bologna, Italy.

[4] Farmitalia Carlo-Erba, Milano, Italy.

**Reprint requests to:* Dr. Franco Pannuti, Divisione di Oncologia, Ospedale M. Malpighi, Via Albertoni 15, 40138 Bologna, Italy.

MATERIALS AND METHODS

Patients

Twenty-two patients with solid tumors who were admitted to the Oncology Division of Ospedale M. Malpighi, Bologna, Italy, were studied. All patients had received prior chemotherapy and/or irradiation or hormonotherapy. These oncologic treatments were discontinued at least 1 month before, and any other treatment 2 days before, the start of the study.

Eleven patients had normal hepatic and renal functions, five had impaired kidney functions (elevated serum creatinine and BUN), and six had liver metastasis and showed abnormalities in serum liver function tests (bilirubin, SGOT, SGPT, and alkaline phosphatase).

Chemicals

4′-EpiDX·HCl for clinical use and the pure analytic standards of 4′-epiDX, 4′-epiDXol, and daunorubicin were supplied by Farmitalia Carlo-Erba (Milan, Italy). Other chemicals were supplied by Merck (Darmstadt) or Farmitalia Carlo-Erba (Milan) and were of analytic grade.

Drug Administration

4′-EpiDX was administered as a rapid iv infusion over a 1–2-min period. The dose administered ranged from 35.7 to 90 mg/m^2. As a rule, the highest dose was given to patients with normal hepatic and renal functions.

Blood Samples

Blood samples (4 ml) were drawn at 0, 15, 30, and 60 mins and at 2, 3, 4, 6, 8, 10, 24, 48, 72, and 96 hrs after drug administration. Samples were immediately centrifuged and the separated plasma was kept frozen at −20°C until analysis.

Drug Analysis

4′-EpiDX and 4′-epiDXol in plasma were specifically determined by a highly sensitive high-pressure liquid chromatography method.

Extraction Procedure

To the plasma samples in silanized centrifuge tubes were added 100 µl of a stock solution of daunorubicin hydrochloride (as internal standard; about 1 µl/ml), 1 ml of phosphate buffer, and 10 ml of chloroform:heptanol (9:1). The mixture was shaken for 20 mins (horizontal shaker, 120 impulses/min) and then centrifuged at 4000 rpm for 10 mins. The organic layer was collected and extracted with 0.5 ml of 0.3 M phosphoric acid in a vortex mixer. The acidic phase, after treatment with 2 ml of *n*-hexane to remove nonpolar contaminants, was analyzed by high-pressure liquid chromatography (10–100-µl injections).

Chromatographic Analysis

Chromatographic analysis was performed with a Perkin Elmer series 3B liquid chromatograph equipped with a 650-10 LC fluorescent detector (470-nm excitation wavelength, slit width 10 nm; 580-nm emission wavelength, slit width 20 nm). In addition, a Supelcosil LC-18 column (15 cm × 4.6 mm internal diameter) was used, and quantitation was performed with a Perkin Elmer Sigma-10 data system. The mobile phase consisted of methanol:water (3:1) plus 0.5% acetic acid and 2.5 mM sodium pentane sulphonate. Several authors reported considerable separation for DX and daunorubicin when using reversed-phase columns and methanol or acetonitrile and phosphate buffer as the mobile phase. We found that the use of paired ion chromatography improves the peak shapes and reduces the analysis time. The method was calibrated in the usual way, with standards made by supplementing blood bank plasma with stock solutions of the drug. The response was linear in the experimentally observed range of concentrations. The coefficient of variation on replicate analyses was 8% for plasma levels ⩾ 3 ng/ml.

Pharmacokinetic Analysis

Visual inspection of the plasma-level profile of unchanged 4′-epiDX shows that a triexponential curve adequately describes the pattern of plasma-level decreases according to the equation

$$Cp\,(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + C \cdot e^{-\gamma t}.$$

The values of the parameters of the triexponential function were determined by submitting the individual plasma-level curves to a nonlinear least-squares fit computer program (MINUIT, CERN Library) and a multistage program using both SIMPLEX (9) and a Davidson and Fletcher algorithm (10). Each minimization process was followed by an extensive search of other possible local minima and the analysis of the "error hole" map (11).

Calculated curves fit closely to experimental values within the time interval investigated (15 mins to 96 hrs); for some patients, abnormally high plasma levels were extrapolated in the initial interval (0–15 mins). As a consequence of these extrapolation problems, a reliable estimation of some parameters in terms of the conventional three-compartment open model cannot be safely made. Therefore, only the slow disposition rate with the corresponding biologic terminal half-life, the area under the plasma-level curve (AUC), the plasma clearance (Pl Cl = dose/AUC), and the apparent volume of distribution at pseudoequilibrium (Vd = Pl Cl/γ) were taken into account in this discussion. The AUC ($0-\infty$) was estimated by adding AUC (15 mins to ∞, calculated from the computer-fitted curve) to the rectangular area in the interval 0–15 mins (with height equal to the plasma level measured at 15 mins).

To compare the pharmacokinetic properties of the drug in different patients, AUC was linearly corrected for each dose administered. For the metabolite 4′-epiDXol, the pharmacokinetic parameters taken into account were the AUC (calculated by trapezoidal rule) up to 96 hrs and the ratio between the AUC of the metabolite and the AUC of the unaltered drug.

RESULTS

The time course of 4′-epiDX and 4′-epiDXol plasma levels after iv administration of 90 mg/m^2 to patients with normal renal and hepatic functions is shown in figure 1 (semilog plot, mean values of nine patients). The plasma-level profile of 4′-epiDX appears to be triexponential, with initially very rapid decrease (lasting 1–2 hrs) and a slow terminal linear phase (established 24 hrs after drug administration). Mean plasma levels of 4′-epiDXol were substantially lower than those of the parent compound over the whole time interval investigated. The plasma-level curves of the two compounds were nearly parallel during the terminal linear phase.

The individual values of the 4′-epiDX kinetic parameters in patients with normal hepatic and renal functions are listed in table 1. The mean terminal plasma half-life was about 40 hrs (coefficient of variation [CV] = 49%), indicating a slow elimination of the drug, in spite of its very efficient mean Pl Cl of about 0.88 L/min (CV = 28%). The apparent discrepancy between the slow elimination and the very efficient clearance process can be explained by an extensive partition of the drug in the tissues, suggested by the very high apparent Vd (46 L/kg, CV = 54%). As regards 4′-epiDXol, its AUC was about 25% (CV = 39%) of that of its parent compound.

The parallelism between the plasma-level curves of the two compounds during the linear terminal phase suggests that the rate constant for metabolite elimination is at least as large as that of the unaltered drug (12). No substantial differences were detected between the first and the fourth treatment courses. In the two patients who were studied on both occasions, the pa-

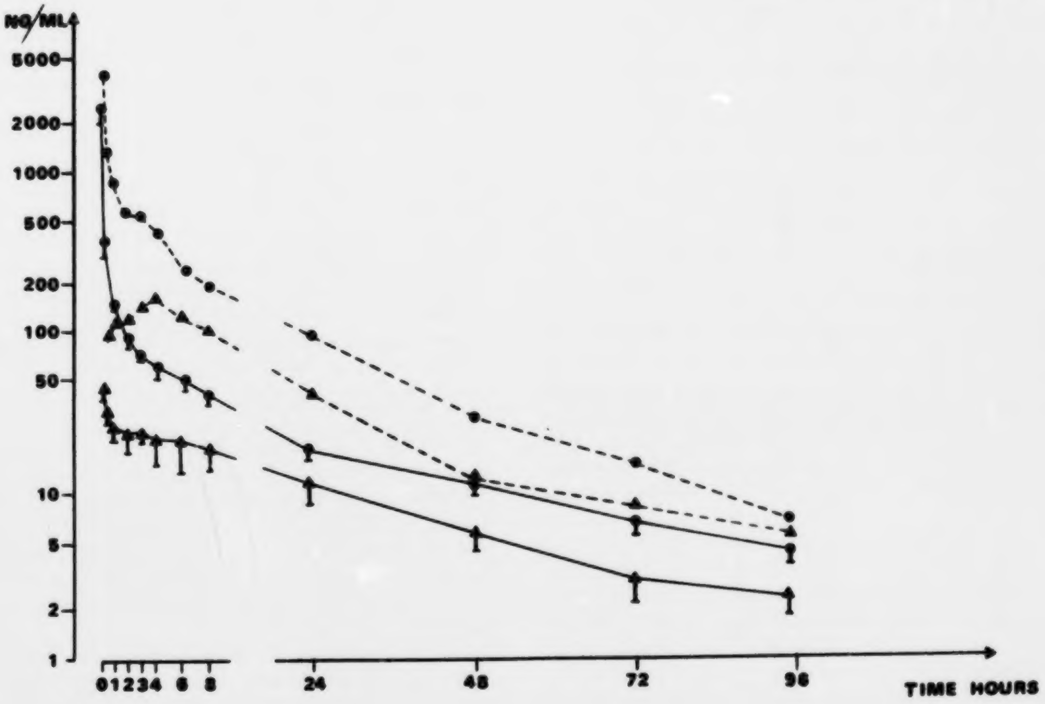

FIGURE 1.—4′-EpiDX (●) and metabolite 4′-epiDXol (▲) concentrations following 90 mg/m^2 iv bolus doses in 9 patients with normal hepatic and renal functions (solid line; mean ± SE) and in patient 17 with hepatic metastases (broken line).

rameters AUC/D and Pl Cl were remarkably similar. The large variations observed in half-life and Vd reflect the high variability of these parameters as reflected in the CV of these terms.

The individual values of kinetic parameters in five patients with renal insufficiency and in six with liver metastases are shown in tables 2 and 3. As with DX treatments, doses were decreased, except in patient 17 (liver metastasis; normal bilirubin level; high values for alkaline phosphatase, SGOT, and SGPT), who received the full dose of 90 mg/m^2. In this patient, 4'-epiDX and 4'-epiDXol plasma levels were consistently higher than in other patients treated with the same dose (fig 1).

The plasma-level profile of the drug and its metabolite in the patients treated with a reduced dose was qualitatively similar to that observed in patients with normal renal and hepatic functions. In patients with renal insufficiency (table 2), the mean AUC corrected for the dose was higher (48.3 vs 34.8) and the mean Pl Cl was lower (0.68 vs 0.88 L/min) than in patients with normal liver and kidney functions. However, examination of individual data reveals that the values for patients with renal impairment are within the same range as those observed in the group with normal renal function in all but one patient (No. 14, whose mean AUC/D and Pl Cl values were about three times higher and one-third lower, respectively, than the mean normal values). No important differences were found in the plasma half-life and Vd.

In the group of patients with liver metastases (table 3), Pl Cl was significantly lower than in patients with normal hepatic function (a mean value of 0.51 vs 0.88 L/min; $P = 0.0065$, Mann-Whitney and Kruskal-Wallis tests) (13), but AUC/D was larger (66.19 vs 34.84; $P = 0.02$, Mann-Whitney and Kruskal-Wallis tests). This high mean AUC/D value is in part due to the value for patient 17, who was treated with a normal dose. As for the other pharmacokinetic parameters (terminal plasma half-life, Vd γ, and AUC ratio), the differences between the two groups were negligible and without statistical significance. In particular, the half-life was not increased.

It is of particular interest that three patients without apparent hepatic lesions and with normal bilirubin levels had high AUC/D values and low Pl Cl values. These patients actually had rather high levels of alkaline phosphatase (patients 1 and 10) or transaminases (patient 5).

No clear-cut correlation between AUC/D or Pl Cl value and any single liver function test was found; however, all possible subset regression analysis (BMDP 9R program) showed a significant ($P = 0.0013$, ANOVA) multiple correlation between AUC/D and levels of SGOT and bilirubin (13).

Hematologic toxicity was mild to moderate and was usually reversible within 3 weeks after drug administration. No correlation was found between the AUC and the wbc count nadir. Only four of 22 patients had a wbc count nadir $< 2 \times 10^3$/mm^3, with the lowest value (0.5×10^3/mm^3) observed in the patient (No. 17) with liver metastases treated with the full dose of 90 mg/m^2.

Of 12 patients evaluable for response, four had partial remissions (patients 4, 5, 10, and 11) while two had minimal remissions (patients 9 and 17).

DISCUSSION

The results of this study confirm that the disposition of 4'-epiDX after administration in man is qualitatively similar to that of DX. The similarities include the typical triexponential plasma level profile of the unaltered drug, having a very long terminal plasma half-life, as well as the time course of the plasma levels of the reduced 13-hydroxy metabolite (14-17). This disposition is interpreted in terms of the classical multicompartment model, which has a high-capacity deep compartment, primarily responsible for the slow elimination of the drug.

Pharmacokinetic parameters demonstrated a rather broad interpatient spread, particularly evident in the terminal plasma half-life and Vd γ (CV of about 50%). Among patients with impaired and normal renal function, no significant differences were found in the disposition of either the drug or its metabolite. As with DX, renal excretion of 4'-epiDX and 4'-epiDXol comprises only a small part of the total elimination of both compounds, so dose modification is not mandatory if renal failure occurs.

On the contrary, both a statistically significant increase of the AUC (corrected for the dose) and a decrease of Pl Cl were found in the group of patients with liver metastases, in accordance with the predominant hepatic elimination of the drug. The terminal plasma half-life remained within the range of values observed in patients with normal liver function; this agrees with the findings of Perrier and Gibaldi (18), who demonstrated that clearance and not the long half-life term is a direct measure of the intrinsic metabolic activity of the liver, if the liver is an integral part of the central compartment.

No evidence was found of either altered formation or altered elimination of 4'-epiDXol, which contradicts the results of a pharmacokinetic study using DX in hepatoma patients (19). This finding suggests that a reduction to about 50% of the usual dose of 4'-epiDX is necessary to reduce to a minimum the possibility of aggravated toxicity (in cancer patients with liver metastases).

The reduced dose of 4'-epiDX prevented any strong hematopoietic suppression in five patients with liver metastases, even in the two having very high bilirubin levels (5.2 and 10.8 mg/100 ml), but severe leukopenia (0.5×10^3 wbcs/mm^3) occurred in the patient (No. 17)

TABLE 1.—Main pharmacokinetic parameters of 4′-epiDX in patients with normal hepatic and renal functions*

Patient No.	Dose		AUC/D†	Pl Cl, L/min	Terminal plasma half-life, hrs	Vd γ, L/kg	AUC for 4′-epiDXol
	mg/m^2	mg					
1	90	170	53.50	0.588	64.6	43.8	0.508
2	90	150	35.16	0.948	29.0	26.4	0.207
3	90	160	22.71	1.304	19.0	28.8	0.260
4	70	121	47.57	0.606	34.4	43.3	0.172
5	90	130	41.05	0.586	29.4	30.8	0.282
6	90	150	25.41	1.093	14.3	20.5	0.208
7	90	145	28.20	0.952	75.1	99.3	0.220
	(60)	(100)‡	(23.91)	(1.161)	(24.2)	(39.0)	(0.156)
8	90	150	26.96	1.030	30.8	46.1	0.214
	(90)	(150)‡	(23.54)	(1.179)	(42.5)	(73.0)	(0.091)
9	90	160‡	27.81	1.065	57.0	77.4	0.147
10	90	140‡	44.20	0.586	30.7	25.7	0.341
11	60	150‡	30.70	0.950	55.4	65.1	0.224
		Mean	34.84	0.882	40.0	46.1	0.253
		SE	3.08	0.075	5.95	7.49	0.030
		CV	29.33	28.40	49.4	53.9	39.3

*Values in parentheses were excluded from calculation of the mean.

†$\text{AUC/D} = \frac{(\text{ng} \times \text{hr})/\text{ml}}{\text{mg/m}^2}$.

‡4th treatment course.

TABLE 2.—Main pharmacokinetic parameters of 4′-epiDX in patients with impaired renal function

Patient No.	Dose		AUC/D*	Pl Cl, L/min	Terminal plasma half-life, hrs	Vd γ, L/kg	AUC for 4′-epiDXol
	mg/m^2	mg					
12	50	75	41.40	0.603	22.3	21.2	0.163
13	50	85	34.42	0.823	31.2	28.2	0.311
14	50	80	95.98	0.278	32.0	12.3	0.094
15	35.7	50	49.44	0.472	84.9	82.6	0.150
16	50	75	20.52	1.218	24.9	48.6	0.233
		Mean	48.35	0.678	39.1	38.6	0.190
		SE	12.82	0.161	11.6	12.5	0.037

*$\text{AUC/D} = \frac{(\text{ng} \times \text{hr})/\text{ml}}{\text{mg/m}^2}$.

TABLE 3.—Main pharmacokinetic parameters of 4′-epiDX in patients with liver metastasis

Patient No.	Dose		AUC/D*	Pl Cl, L/min	Terminal plasma half-life, hrs	Vd γ, L/kg	AUC for 4′-epiDXol
	mg/m^2	mg					
17	90	135	103.93	0.240	21.1	8.4	0.362
18	70	95	34.18	0.661	31.2	42.5	0.180
19	50	80	47.04	0.567	51.8	41.6	0.200
20	50	85	125.26	0.226	29.6	9.2	0.468
21	50	74	58.34	0.423	29.5	19.5	0.318
22	50	80	28.44	0.937	28.5	41.3	0.187
		Mean	66.19	0.509	31.9	27.1	0.285
		SE	16.11	0.111	4.2	6.8	0.047

*$\text{AUC/D} = \frac{(\text{ng} \times \text{hr})/\text{ml}}{\text{mg/m}^2}$.

who was treated with 90 mg/m^2. However, this patient, who had normal bilirubin levels (0.8 mg/100 ml), was exposed to very high 4′-epiDX plasma levels (three to four times higher than those in normal patients treated with the same dose, in terms of AUC). The results of the present study do not permit any conclusion regarding the relationship between impaired liver function, 4′-epiDX pharmacokinetics, and drug effects. However, in view of both the previously discussed findings and the similar disposition of 4′-epiDX and DX, dose modification should be recommended in patients with signs of liver disorders as well as in those with liver metastases.

Further studies are necessary to elucidate the fate of 4′-epiDX after administration in man, with particular reference to the routes of excretion, the metabolic pathway, and the relative contributions of metabolism and biliary and renal excretion to the drug clearance. Such knowledge will assist in selecting the particular tests of liver function that can best account for major impairment in the clearance process of the drug and can predict the changes in drug pharmacokinetics.

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Modulation of Cytarabine Metabolism in the Human Promyelocytic Leukemia Cell Line HL-60 by Polyhydroxy-Substituted Benzohydroxamic Acids[1,2]

Stephen B. Howell,[2,3,*] Susan Gill,[3] and Howard L. Elford[4,5]

Two potent new ribonucleotide reductase inhibitors, 3,4,5-trihydroxybenzohydroxamic acid (VF 122) and 3,4-dihydroxybenzohydroxamic acid (VF 147), were investigated for their ability to modulate the cellular pharmacology of cytarabine (ara-C) in HL-60 cells. VF 122 and VF 147 increased the total cellular uptake of ara-C by a mean (± SE) of 8% ± 3% and 29% ± 3%, respectively, when measured 2 hours after the start of exposure to 0.1 μM ara-C. This effect was evident after only 10 minutes of exposure to the ribonucleotide reductase inhibitor and did not vary significantly over the concentration range of 10–100 μM for either agent. VF 122 enhanced the incorporation of ara-CTP into DNA by 3.6-fold; VF 147 produced a 5.6-fold increase. In comparison, the maxiumum enhancement achievable with hydroxyurea was 2.1-fold, and with thymidine was 1.8-fold. These results provide a biochemical rationale for further investigation of these agents in combination with ara-C. [Cancer Treat Rep 66:1825–1829, 1982]

Cytarabine (ara-C) is one of the most important drugs currently available for the treatment of acute leukemia in man (1). The phosphorylation of ara-C by dCyd kinase is the rate-limiting step in the conversion of ara-C to its metabolite ara-CTP (2). Ara-CTP is a potent inhibitor of DNA polymerase in vitro (3,4) but in whole cells is also incorporated into DNA (5–9). The cytotoxicity of ara-C does not correlate well with its ability to inhibit DNA synthesis (10–13). However, it does correlate well with the amount of ara-C incorporated into DNA (8,9), and with the production of chromatid breaks (10,11). The extent of ara-CTP incorporation into DNA should be a function of the amount of ara-CTP formed, and recent work on the cellular pharmacology of ara-C in freshly harvested leukemic blasts demonstrated a correlation between the clinical response to ara-C and the extent of formation and retention of ara-CTP (14).

The effectiveness of ara-C is also influenced by the concentration of the normal substrate dCTP, with which ara-CTP must compete for binding to DNA polymerase (3,4,15,16). Phosphorylation of ara-C is normally constrained by feedback inhibition by dCTP (2,17). Theoretically, any drug that can reduce the intracellular concentration of dCTP should simultaneously enhance the phosphorylation of ara-C, increase the effectiveness of ara-CTP in inhibiting DNA polymerase, and increase the probability that ara-C rather than dCyd will be incorporated into DNA. One technique for accomplishing this is to inhibit the enzyme ribonucleotide reductase, which carries out an obligatory step in the de novo synthesis of dCTP (18).

Both thymidine (dThd) and hydroxyurea (HU) are inhibitors of ribonucleotide reductase (18–22). Both have been shown to increase the incorporation of ara-CTP into DNA (2,23,24) and to interact synergistically with ara-C with respect to cytotoxicity (23,25–30). Recently, Van't Reit et al (31) have synthesized a series of polyhydroxy-substituted benzohydroxamic acids which are 20–50 times more potent than HU as inhibitors of ribonucleotide reductase. The aim of this research was

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[1] Received Jan 4, 1982; revised July 12, 1982; accepted July 21, 1982.

[2] Supported in part by Public Health Service grant CA-25336 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Clayton Foundation for Research, California Division. Dr. Howell is a Clayton Foundation Investigator.

[3] Department of Medicine and the Cancer Center, University of California-San Diego, La Jolla.

[4] Virginia Associated Research Campus, The College of William and Mary, Newport News.

[5] We thank Dr. Bart Van't Reit of the Medical College of Virginia for supplying the hydroxy-substituted benzohydroxamic acids, and for providing the impetus for this study.

[*] *Reprint requests to:* Stephen B. Howell, MD, Department of Medicine T-006, University of California-San Diego, La Jolla, CA 92093.

to determine whether even greater enhancement of ara-CTP incorporation into DNA could be achieved with these new agents.

MATERIALS AND METHODS

Cell Lines

HL-60 cells obtained from Dr. S. J. Collins were seeded at 5 × 10^5 cells/ml in RPMI-1640 medium with 10% heat-inactivated fetal calf serum, 1% antibiotic solution (penicillin at 10,000 units/ml, fungizone at 25 μg/ml, and streptomycin at 10,000 μg/ml; Flow Laboratories, McLean, VA), and 1% glutamine at 37°C in 5% CO_2. The cultures were allowed to grow for 1 day to 0.9–1.0 × 10^6 cells/ml before being used in experiments.

Drugs

The two compounds, 3,4-dihydroxybenzohydroxamic acid (VF 147) and 3,4,5-trihydroxybenzohydroxamic acid (VF 122), were obtained from Dr. Bart Van't Reit, Medical College of Virginia, Richmond.

Cells (10^6/ml) were exposed to 10–100 μM VF 122 and VF 147 for varying times prior to the start of exposure to 0.1 μM ^{3}H-ara-C ([5-^{3}H]1-β-D-arabinofuranosylcytosine, 10.2 Ci/mmol; New England Nuclear, Boston). After the start of ^{3}H-ara-C exposure, aliquots of cells were removed at various times, washed twice with ice-cold phosphate-buffered saline, and precipitated with perchloric acid, and then both the acid-soluble and acid-insoluble fractions were counted by liquid scintillation. Incorporation of ^{3}H-ara-C into ara-CTP was determined by extracting 1-ml aliquots of cells with 0.4 M perchloric acid, neutralizing the extract with alamine and freon, and then separating ara-CTP from other forms of ara-C on polyethylene amine thin-layer chromatographic sheets (Brinkman, Westbury, NY). The sheets were run in 50% methanol, dried, and then run in 1 M LiCl. Labeled ara-CTP spots were located by UV light after adding cold ara-CTP (Calbiochem, San Diego). The ara-CTP spots were then cut out and counted in a liquid scintillation counter.

The extent of ^{3}H-ara-C incorporation into DNA was measured using the technique described by Kufe et al (8). Ten million cells (10^6/ml) were exposed to 100 μM VF 122 or VF 147 for 30 minutes and then simultaneously exposed to the ribonucleotide reductase inhibitors plus 0.1 μM ^{3}H-ara-C and 10 μCi/ml of $^{32}PO_4$ (carrier free, New England Nuclear) for an additional 2 hours. The cells were then digested with pronase B (Calbiochem) and extracted with phenol. The DNA was separated from RNA by banding the extract on a cesium sulfate gradient. Trichloroacetic acid precipitates of 1-ml aliquots were counted by liquid scintillation.

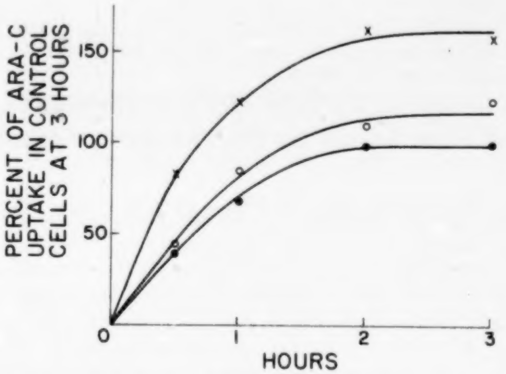

FIGURE 1.—Effect of 100 μM VF 122 (○) and VF 147 (×) on the total cellular uptake on ^{3}H-ara-C in HL-60 cells. Data are expressed as % of the uptake in control cells (●) after a 3-hr incubation with media alone. Cells were pretreated with VF 122 and VF 147 for 30 mins prior to the start of exposure to 0.1 μM ^{3}H-ara-C.

RESULTS

A concentration of 110 μM VF 122 reduced the growth rate of HL-60 cells by 50%; the corresponding concentration of VF 147 was 44 μM. The effect of each of these agents on the cellular uptake of ara-C and on the rate of incorporation of ara-CTP into DNA was studied at approximately equitoxic concentrations and compared to that of HU and dThd.

Figure 1 shows the effect of optimal concentrations of VF 122 and VF 147 on the total cellular uptake of ^{3}H-ara-C as a function of time. The ara-C concentration was 0.1 μM, a concentration commonly achieved by constant infusion during the treatment of patients with acute leukemia (33). For both of these drugs, as for HU and dThd (data not shown), the rapid phase of ^{3}H-ara-C uptake was complete by 2 hours. When HL-60 cells were preincubated with ribonucleotide reductase inhibitor at the optimal concentration for 30 minutes prior to the start of ^{3}H-ara-C exposure, VF 122 increased uptake by a mean (± SE) of 8% ± 3% at 2 hours. VF 147 produced a mean (± SE) increase of 29% ± 3% under the same conditions. In contrast, even without the 30-minute preincubation, HU and dThd produced a 300%–400% increase in ara-C uptake (34) at their optimal concentrations. Both VF 122 and VF 147 were tested at concentrations of 10–100 μM, but there was no significant variation in their effect on the cellular metabolism of ^{3}H-ara-C over this range.

Enhancement of ara-C uptake by HL-60 cells may be due to either an increase in conversion of ara-C to ara-CTP and incorporation into DNA or accumulation of cells in S phase. Although HU and dThd cause synchronization of cells in S phase, and a similar effect is expected from VF 122 and VF 147 based on their

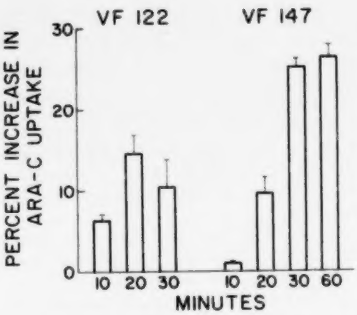

FIGURE 2.—Effect of the duration of pretreatment of HL-60 cells with 100 μM of VF 122 (left) and VF 147 (right) on the rate of total cellular ara-C uptake over the 1st 30 mins following the start of exposure to 0.1 μM ^{3}H-ara-C. Vertical bars = SD.

mechanisms of action, the data presented in figure 2 effectively exclude synchronization as an explanation for the change in the cellular metabolism of ara-C. The doubling time of HL-60 is 24 hours, whereas the effect of both VF 122 and VF 147 was apparent within 10 minutes of the start of drug exposure.

Table 1 shows the influence of each agent on the rate of ara-CTP incorporation into DNA at a concentration of the ribonucleotide reductase inhibitor which enhanced ara-C uptake. All four agents slowed the rate of DNA synthesis as evidenced by a reduction in the rate of incorporation of $^{32}PO_4$ into DNA. Despite this, all four agents increased the rate of ara-CTP incorporation per unit of newly synthesized DNA. The values given for the degree of enhanced incorporation for VF 122, VF 147, and dThd are for the optimal concentration of each agent; the degree of enhancement of ara-C uptake produced by HU continued to increase at HU concentrations > 1000 μM. Thus, despite the fact that VF 122 and VF 147 were not as effective as HU and dThd at increasing the cellular uptake of ara-C, they were more effective in promoting the incorporation of ara-CTP into DNA.

DISCUSSION

The polyhydroxy-substituted benzohydroxamic acids are primarily of interest because they are potent inhibitors of ribonucleotide reductase. When tested against ribonucleotide reductase from the Novikoff hepatoma, VF 122 was 50-fold and VF 147 was 20-fold more potent than HU (35). In the murine L1210 leukemia system, both drugs were more potent than HU and were at least as effective as HU in improving the lifespan of treated animals (35). In previous investigations in this laboratory, we have established that both dThd and HU can modulate the cellular pharmacology of ara-C to increase its incorporation into DNA, and that the mechanism of this effect in HL-60 cells is a marked reduction in the size of the dCTP pool due to inhibition of ribonucleotide reductase (34). The results of this study indicate that VF 122 and VF 147 are also capable of enhancing the cellular uptake of ara-C and the incorporation of ara-CTP into DNA. Although we have not specifically investigated the effects of VF 122 and VF 147 on the ribonucleotide reductase from HL-60 cells, there is nothing in the data that is inconsistent with the hypothesis that VF 122 and VF 147 are modulating the cellular metabolism of ara-C via inhibition of this enzyme.

A comparison of all four ribonucleotide reductase inhibitors points out an interesting dissociation between their effect on total cellular uptake and incorporation into DNA. HU and dThd, at their optimal concentrations, produced a doubling of the incorporation per unit of newly synthesized DNA (34). In contrast, VF 122 and VF 147 produced a greater effect on the incorporation of ara-CTP into newly synthesized DNA, but only an 8% and 29% increase, respectively, in total cellular uptake of ara-C. In the case of dThd and HU we have previously reported that the increase in total cellular uptake of ara-C is very closely matched by an increase in the ara-CTP pool size (34). We have not investigated the effect of VF 122 and VF 147 on the relative ara-CTP and dCTP pools of HL-60 cells; however, it has been shown that these compounds will lower the pools of dCTP in HeLa cells (36). It is possible that these drugs affect the distribution of total cellular ara-C among its various phosphorylated metabolites. To our knowledge, no information is available on the effect of these compounds on deoxycytidylate deaminase, whose activity can potentially influence the incorporation of

TABLE 1.—Effect of ribonucleotide reductase inhibitors on incorporation of ^{3}H-ara-C and $^{32}PO_4$ into DNA

Ribonucleotide reductase inhibitor	% of control ^{3}H-ara-C incorporated into DNA	% of control $^{32}PO_4$ incorporated into DNA	Ratio of ^{3}H-ara-C/$^{32}PO_4$ incorporated into DNA
VF 122 (100 μM)	163	45	3.6
VF 147 (100 μM)	175	31	5.6
HU (1000 μM)	25	14	2.1
dThd (1000 μM)	129	62	1.8

ara-CTP into DNA by converting ara-CMP to ara-UMP (37–40).

The major significance of the ability of VF 122 and VF 147 to enhance the incorporation of ara-CTP into DNA lies in the recent demonstration by Kufe and his colleagues (8) and Major et al (9) that the cytotoxicity of ara-C correlates very well with DNA incorporation in HL-60 cells and freshly harvested acute leukemic myeloblasts. Using the same technique and cell line as used in this study, they have shown that ara-C is incorporated into internucleotide linkage as ara-C rather than ara-U or other metabolites (9). On the other hand, it has been known for some time that the cytotoxicity of ara-C does not correlate well with its ability to inhibit DNA synthesis (10–13). We and others (23,25–30) have previously reported that dThd and HU are synergistic with ara-C in various systems at concentrations that increase ara-C incorporation into DNA. It remains to be demonstrated that VF 122 and VF 147 behave the same way, but if so, they may find utility in combination with ara-C for the treatment of acute leukemia.

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Antiemetic Activity of Butorphanol Against Cisplatin-Induced Emesis in Ferrets and Dogs[1]

John E. Schurig,* Alexander P. Florczyk, William C. Rose, and William T. Bradner[2,3]

The analgesic agent butorphanol was evaluated for its ability to block cisplatin-induced emesis in ferrets and dogs. In ferrets, butorphanol (0.15, 0.3, or 0.45 mg/kg; expressed in terms of the tartrate salt) administered sc 30 minutes prior to cisplatin (8 mg/kg iv) reduced the number of emetic episodes but did not eliminate them. When these doses of butorphanol were administered 30 minutes before and 30 and 90 minutes after cisplatin, they caused a dose-related reduction in the number of emetic episodes; there was complete protection at a dose of 0.45 mg/kg/injection. In dogs, butorphanol (0.185 or 0.37 mg/kg/injection, expressed in terms of the tartrate salt) was administered sc on the same multiple-dose schedule used in the ferrets; this caused a nearly complete elimination of the emetic response to cisplatin (3 mg/kg iv). Butorphanol caused some sedation in both species at effective antiemetic doses but had no effect on the antitumor activity of cisplatin against murine L1210 leukemia. Naloxone blocked the antiemetic effect of butorphanol in ferrets, indicating the involvement of opiate receptors. The results of these studies suggest that butorphanol may be useful clinically in mitigating the emetic effects of cisplatin. [Cancer Treat Rep 66:1831–1835, 1982]

The nausea and vomiting induced by cancer chemotherapeutic agents is a common and distressing problem for cancer patients. Prolonged nausea and vomiting can have serious consequences on nutrition, metabolism, and the quality of life of such patients and has caused some of them to reject potentially lifesaving drug treatment (1–3). The currently available antiemetics such as the phenothiazines, antihistamines, and butyrophenones offer some relief but are generally considered to be inadequate against the more emetogenic chemotherapeutic agents (4–7). Consequently, there is a great deal of interest in the development of new antiemetic drugs that are more effective against cancer chemotherapeutic agents.

Previous studies by Florczyk et al (8) and Gylys et al (9) have shown that ferrets and dogs vomit repeatedly beginning approximately 60–90 minutes after cisplatin administration. We now report the results of our evaluations of the antiemetic effects of butorphanol, an agonist-antagonist analgesic agent (10), against cisplatin-induced emesis in these animals. We also evaluated the effect of butorphanol on the antitumor activity of cisplatin against murine L1210 leukemia.

MATERIALS AND METHODS

Animals

The antiemetic studies were performed in conscious male Fitch ferrets (1–1.5 kg) and beagle dogs of either sex (9–12 kg). To facilitate iv dosing, each ferret had an indwelling jugular catheter surgically implanted 2–4 days prior to use, as previously described (11). Female CDF_1 mice (16–19 g) were used in the antitumor studies. All animals were given free access to feed and water.

Antiemetic Studies

In ferrets, butorphanol (0.15, 0.3, or 0.45 mg/kg/injection) was injected sc in the scapular region 30 minutes prior to the administration of cisplatin (8 mg/kg iv). Additional doses of butorphanol were administered sc 30 and 90 minutes after cisplatin in the multiple-dose

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[1] Received Dec 30, 1981; revised June 14, 1982; accepted July 12, 1982.

[2] Department of Antitumor Biology, Bristol Laboratories, Syracuse, NY.

[3] We gratefully acknowledge the contributions of Drs. J. Vida and A. Pircio and the expert technical assistance of S. Putzer. We thank S. Bennett and R. Sutliff for typing the manuscript.

**Reprint requests to:* Dr. John E. Schurig, PO Box 657, Bristol Laboratories, Syracuse, NY 13201.

studies. One group of ferrets was treated with butorphanol (0.45 mg/kg sc) 30 minutes prior to cisplatin and then with naloxone (2 mg/kg sc) 45 minutes after cisplatin. In dogs, butorphanol (0.185 or 0.37 mg/kg/injection) was injected sc in the scapular region 30 minutes before and 30 and 90 minutes after the administration of cisplatin (3 mg/kg iv). The emetic responses of cisplatin, consisting of retching and expulsion as described (in cats) by McCarthy and Borison (12), were determined in both species by direct observation of the animals during a period lasting 4–6 hours after cisplatin administration. The onset time of the first emetic episode and the number of episodes were recorded for each animal. A subjective assessment (minimal, mild, moderate, or marked) was made of any behavioral effects of butorphanol.

Antitumor Studies

CDF$_1$ mice were implanted ip with 10^6 L1210 leukemia cells derived from ascites fluid produced in DBA/2 mice. On Day 1 after implant, the mice (six per group) received either a single ip injection of cisplatin, three sc injections of butorphanol 1 hour apart, or both drugs. When both drugs were administered, butorphanol was given sc 30 minutes before and 30 and 90 minutes after ip cisplatin. The effect of each drug treatment was judged on the basis of the relative increases in median survival time (MST) compared to MST in untreated control mice; this parameter was expressed as % T/C, which represents the MST of a drug-treated group divided by the MST of the control mice, × 100. The doses were determined according to the mean body weight of each group of mice. Cisplatin was administered at 12-, 8-, 6-, and 4-mg/kg dose levels, and butorphanol was administered at doses ranging from 0.6 to 60 mg/kg/injection.

Drugs

In the antiemetic studies, butorphanol was administered as the clinically used parenteral solution (Stadol) or as 0.9% NaCl dilutions of this solution. For the antitumor studies the butorphanol tartrate salt was dissolved in 0.9% NaCl. The doses of butorphanol in all studies were expressed in terms of the tartrate salt. The cisplatin solutions used in the antiemetic studies, 4 mg/ml for ferrets and 3 mg/ml for dogs, were prepared by adding heated (70°C) 0.9% NaCl to the cisplatin, stirring and sonicating until the cisplatin was in solution, and then maintaining the solution at 40°C for administration within 30 minutes. For the antitumor studies, cisplatin was dissolved in 0.9% NaCl without heating, since the concentrations used were ≤ 1 mg/ml. Naloxone was dissolved in 0.9% NaCl and the dose was expressed in terms of the HCl salt.

RESULTS

The results of the single-dose butorphanol studies in ferrets are summarized in table 1. In the control animals (no butorphanol treatment), cisplatin caused 7.4 ± 0.8 (mean ± SE) emetic episodes beginning about 70 minutes after administration. Butorphanol was administered only once, 30 minutes prior to cisplatin. The ferrets pretreated with butorphanol (0.15 and 0.3 mg/kg) had about one-half the number of emetic episodes as the control animals, with some apparent reduction in onset time of emesis; however, there was considerable variability in the number of episodes at these doses, with one of the four animals that received a dose of 0.3 mg/kg having no emesis. The ferrets pretreated with butorphanol at a dose of 0.45 mg/kg had a marked reduction in the number of emetic episodes (0.9 ± 0.5), with five of eight animals experiencing complete protection. The other three ferrets had 2.7 ± 0.3 emetic episodes, which is about one-third the number in the control animals. The behavioral effects of butorphanol consisted of minimal to mild sedation in some animals, which was not dose-related and was difficult to assess reliably due to the normally sedentary behavior of the ferret during daylight hours.

TABLE 1.—Antiemetic activity of single-dose butorphanol tartrate against cisplatin-induced emesis in ferrets

Butorphanol tartrate (mg/kg sc)*	No. with emesis/No. tested	No. of episodes†	Emesis onset in responding animals (mins)†
Control	12/12	7.4 ± 0.8	66.8 ± 3.9
0.15	4/4	3.3 ± 1.1‡	47 ± 5.4‡
0.30	3/4	3.5 ± 2.2	41.7 ± 0.9‡
		(4.7 ± 2.7)	
0.45	3/8	0.9 ± 0.5‡	81 ± 4.6
		(2.7 ± 0.3)‡	

*Single dose administered 30 mins prior to cisplatin (8 mg/kg iv).

†Values = mean ± SE. Values in parentheses = No. of episodes in responding animals.

‡$P < 0.05$, significantly different from control; Student's t test.

A summary of the results from the multiple-dose butorphanol studies is shown in table 2. The controls from the single- and multiple-dose studies were pooled since many of the tests were done concurrently. Therefore, the control data in table 2 are the same as in table 1. Butorphanol administered on the multiple-dose schedule caused a dose-related reduction in the number of emetic episodes, with complete protection in one animal at a dose of 0.3 mg/kg/injection and in all animals at a dose of 0.45 mg/kg/injection. There was essentially no effect on onset time of emesis in the animals responding to cisplatin. The behavioral effects of butorphanol observed in these studies consisted of sedation, which was minimal at the low dose, mild at the intermediate dose, and mild to moderate at the high dose.

The results of the evaluation of the antiemetic activity of butorphanol in dogs are shown in table 3. Cisplatin (3 mg/kg) caused emesis in the control dogs, with a mean of 14 ± 3 emetic episodes beginning about 100 minutes after dosing. Butorphanol administered at a dose of 0.185 or 0.37 mg/kg/injection 30 minutes before and 30 and 90 minutes after cisplatin markedly reduced the number of emetic episodes. Only one of the three dogs in each butorphanol dose group vomited, and each had only two episodes. The onset times were comparable or slightly longer than in the control animals. The behavioral effects of butorphanol consisted of minimal to mild sedation at the low dose and mild to marked sedation at the high dose.

Table 4 summarizes the results of a study to determine if butorphanol had any effect on the antitumor activity of cisplatin against murine L1210 leukemia. Cisplatin alone produced a maximum T/C of 171% at a dose of 8 mg/kg ip. Butorphanol administered sc at doses of 0.6–60 mg/kg/injection, on the same multiple-dose schedule used in the emesis studies, had no effect on the antileukemic activity of cisplatin as determined at optimal dose levels, nor did butorphanol exhibit any antileukemic activity of its own.

The results of studies done to evaluate the effect of naloxone on the antiemetic activity of butorphanol against cisplatin-induced emesis in ferrets are shown in table 5. In the control animals, cisplatin caused 6.7 ± 0.5 emetic episodes with an onset time of about 70 minutes. Butorphanol (0.45 mg/kg) administered as a single dose 30 minutes prior to cisplatin completely blocked the emetic effect of cisplatin in all three animals. In the third group of ferrets, butorphanol (0.45 mg/kg) was given 30 minutes prior to cisplatin, and naloxone (2 mg/kg) was given 45 minutes after cisplatin. These ferrets responded to the emetic effect of cisplatin, with an average of 14.5 emetic episodes. The last group of ferrets

TABLE 2.—Antiemetic activity of multiple-dose butorphanol tartrate against cisplatin-induced emesis in ferrets

Butorphanol tartrate (mg/kg/injection sc)*	No. with emesis/ No. tested	No. of episodes†	Emesis onset in responding animals (mins)†	Sedation
Control	12/12	7.4 ± 0.8	66.8 ± 3.9	—
0.15	3/3	3.7 ± 1.7	86.6 ± 27.2	Minimal
0.30	3/4	2.0 ± 0.9‡ (2.7 ± 0.9)	80.7 ± 16.2	Mild
0.45	0/4	0‡	—	Mild to moderate

*Administered 30 mins before and 30 and 90 mins after cisplatin (8 mg/kg iv).
†Values = mean ± SE. Values in parentheses = No. of episodes in responding animals.
‡$P < 0.05$, significantly different from control; Student's t test.

TABLE 3.—Antiemetic activity of butorphanol tartrate against cisplatin-induced emesis in dogs

Butorphanol tartrate (mg/kg/injection sc)*	No. with emesis/ No. tested	No. of episodes†	Emesis onset in responding animals (mins)†	Sedation
Control	3/3	14 ± 3.1	102 ± 10.4	
0.185	1/3	0.7 ± 0.7‡ (2.0)	112	Minimal to mild
0.37	1/3	0.7 ± 0.7‡ (2.0)	150	Mild to marked

*Administered 30 mins before and 30 and 90 mins after cisplatin (3 mg/kg iv).
†Values = mean ± SE. Values in parentheses = No. of episodes in responding animals.
‡$P < 0.05$, significantly different from control; Student's t test.

TABLE 4.—Effect of butorphanol tartrate on antileukemic activity of cisplatin against murine L1210 leukemia*

Butorphanol tartrate (mg/kg sc)	Cisplatin	
	Optimal dose† (mg/kg ip)	Maximum % T/C
0	8	171
0.6	6	171
1.5	8	179
6	8	179
15	6.8	171
60	6	164

*CDF_1 mice were implanted ip with 10^6 L1210 cells. Treatments were given 1 day after implant. Cisplatin was administered ip at doses of 12, 8, 6, and 4 mg/kg. Butorphanol was administered 30 mins before and 30 and 90 mins after cisplatin. There were 6 mice in each group. Butorphanol alone had no antileukemic effect.

†That dose achieving the greatest % T/C at acceptable levels of toxicity (not > 1 of 6 mice dying within 4 days of drug treatment).

received the treatment with butorphanol and naloxone without cisplatin and had no emetic episodes.

DISCUSSION

In these studies, butorphanol had antiemetic activity against cisplatin-induced emesis in both ferrets and dogs. This effect of butorphanol resulted in marked reductions in the number of emetic episodes and in some cases, complete protection. To our knowledge the antiemetic activity of butorphanol has not been described previously and therefore represents a novel effect for an agonist-antagonist type of analgesic agent. It has been shown that the narcotic analgesic agents such as morphine and fentanyl have antiemetic activity in cats and dogs (13,14). However, the narcotic analgesics are not often used clinically as antiemetics in cancer chemotherapy (7). Therefore, butorphanol could represent a new antiemetic agent for addition to the armamentarium used against emesis induced by chemotherapeutic agents. Due to its antagonist properties, however, the use of butorphanol would be restricted to patients having had no prior treatment with analgesics.

The use of ferrets to evaluate drugs for emetic and antiemetic activity is a new approach to this type of testing. Therefore, it was important to confirm the results from the ferret studies in the more widely recognized dog model. In a preliminary report on the use of the ferret as an animal model for emesis testing (8), we showed that the emetic effects of cisplatin in ferrets were similar to those described in dogs (9). This report also showed that metoclopramide, a drug that has antiemetic activity against cisplatin in dogs (9) and man (15,16), was an effective antiemetic in ferrets. The emetic activity of cisplatin and the antiemetic activity of metoclopramide in the animal models and man indicate good correlation between the results in the studies of the ferret and the dog and between the results of the studies of the two animal models and man, relative to their qualitative responses to these two drugs. Because butorphanol had antiemetic activity in both ferrets and dogs, the drug may be expected to have antiemetic activity in man.

The only behavioral effect of butorphanol that was observed in these studies was sedation. This side effect was anticipated since butorphanol can cause sedation in man (17). The degree of sedation appeared to be dose-related in both species in the multiple-dose studies.

An important consideration regarding the use of any toxicity-mitigating treatment in conjunction with a chemotherapeutic agent is the effect on antitumor activity. This is particularly critical with a chemically reactive drug like cisplatin. Butorphanol, at doses ranging

TABLE 5.—Effect of naloxone on the antiemetic activity of butorphanol tartrate against cisplatin-induced emesis in ferrets

Drug	Dose (mg/kg sc)	No. with emesis/ No. tested	No. of episodes*	Emesis onset in responding animals (mins)*
Control	—	7/7	6.7 ± 0.5	69.9 ± 4.0
Butorphanol	0.45†	0/3	0‡	—
Butorphanol + Naloxone	0.45† + 2.0§	2/2	14.5 ± 2.5‡	53.5 ± 2.5‡
Butorphanol + Nalozone¶	0.45 + 2.0	0/2	0	—

*Values = mean ± SE.

†Single dose administered 30 mins before cisplatin (8 mg/kg iv).

‡$P < 0.05$, significantly different from control; Student's t test.

§Single dose administered 45 mins after cisplatin (75 mins after butorphanol).

¶Butorphanol and naloxone administered as single doses, 75 mins apart, without cisplatin.

from 0.6 to 60 mg/kg/injection (30 minutes before and 30 and 90 minutes after cisplatin), had no effect on the antitumor activity of cisplatin against murine L1210 leukemia. Although this was a study of only one tumor type, the results suggest that butorphanol does not interfere with the antitumor activity of cisplatin.

The results with naloxone indicate that opiate receptors are involved with the antiemetic effect of butorphanol. The location of these opiate receptors is not known, but there are data to suggest that they are associated with the vomiting center rather than the chemoreceptor trigger zone (18). Assuming this location is correct, the antiemetic effect of butorphanol may be exerted centrally at the level of the vomiting center. However, additional studies are needed to establish the mechanism of the antiemetic action of butorphanol more adequately.

Naloxone not only blocked the antiemetic effects of butorphanol in ferrets but also appeared to augment the emetic responses to cisplatin (table 5). Treatment with butorphanol followed by naloxone (without cisplatin) was not emetic, indicating that naloxone was not unmasking a butorphanol-induced emesis. This suggests that endogenous opiates may be modulating the severity of the cisplatin emetic response. Additional studies are planned to address this possibility.

Based on results of these studies, butorphanol has become a candidate for clinical trial as an antiemetic agent against cisplatin-induced emesis. In preliminary studies in ferrets (data not shown), we found that iv doxorubicin caused emesis that was blocked by butorphanol. These studies are being expanded, but the initial results indicate that butorphanol has antiemetic activity against emetic cancer chemotherapeutic agents other than cisplatin.

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Response of the Dunning R3327H Prostatic Adenocarcinoma to Radiation and Various Chemotherapeutic Drugs[1]

D. Mador, B. Ritchie, B. Meeker, R. Moore, F. G. Elliott, M. S. McPhee, J. D. Chapman,* and W. H. Lakey[2]

Dunning R3327H prostatic adenocarcinoma was bilaterally transplanted in the flanks of animals at the Papanicolaou Institute in Miami, and the animals were received at the Cross Cancer Institute (Edmonton, Alberta, Canada) each month. The animal flanks were palpated weekly, and when tumor volumes reached a size of approximately 300 mm^3 the animals were randomized into treatment groups for the assessment of various therapies. Tumor volumes were determined each week before and after various treatments, and tumor growth was compared to that in untreated controls. Ionizing radiation at relatively small single doses completely inhibits tumor growth for a period of up to 6 months. Some interesting characteristics of this radiation-induced growth arrest are that tumors do not die and shrink away as with some other tumor models but remain static in size and show histologic evidence of viable tumor cells. The hypoxic cell radiosensitizer misonidazole potentiates radiation response in this tumor model. Cisplatin, vincristine, etoposide, and estramustine phosphate administered in drug doses approaching their toxic limits have a partial effect on tumor growth. [Cancer Treat Rep 66:1837-1843, 1982]

Some physiologic and pathologic aspects of the Dunning R3327H rat prostatic cancer model have been described (1,2). This slow-growing, hormone-responsive tumor has similarities to human prostatic cancer and consequently is of interest in studies designed to test various treatment modalities. Research with this tumor model was initiated at the Cross Cancer Institute (Edmonton, Alberta, Canada) in 1979, and initial studies have involved an evaluation of the utility of this rat prostatic tumor in predicting the response of human prostatic cancer to various treatment modalities. We have measured the effectiveness of ionizing radiation with and without the hypoxic cell radiosensitizer misonidazole and of several chemotherapeutic drugs in current clinical use. All statements on tumor response in these initial experiments with R3327H are based on the effect of treatment on tumor volume growth kinetics.

MATERIALS AND METHODS

First-generation (F_1) hybrids of the matings of the Copenhagen male and Fischer female rats were injected sc in each flank with a 2-mm^3 core of R3327H tumor at the Papanicolaou Institute, Miami. These animals are supplied to various research centers courtesy of the National Prostatic Cancer Project in an effort to standardize treatment results. Implanted rats were flown to the Cross Cancer Institute and examined weekly until the tumor nodule was palpable (approximately 10-16 weeks). Each animal was individually caged and identified throughout the study. The tumor nodules were then measured weekly with calipers in three mutually orthogonal dimensions while the rats were under light halothane anesthesia. A double skin flap dimension of 1.3 mm was subtracted from each caliper reading, and tumor volumes were calculated using the formula $\frac{\pi}{6} \times d_1 \times d_2 \times d_3$, the formula for an ellipsoidal mass.

Animals were randomized into specific treatment protocols when at least one of their tumors reached a volume of 200–400 mm^3. The volumes of at least ten different tumors were averaged to indicate the tumor growth prior to and subsequent to a specific therapy. At least ten different untreated tumors served as normal growth controls at various times throughout the course of these studies, and maximum tumor doubling

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[1] Received Feb 1, 1982; revised June 14, 1982; accepted July 12, 1982.

[2] Departments of Surgery (Urology) and Radiation Oncology (Radiobiology), University of Alberta and Cross Cancer Institute, Edmonton, Canada.

**Reprint requests to:* J. D. Chapman, Department of Radiation Oncology, Cross Cancer Institute, 11560 University Ave, Edmonton, Alberta, Canada T6G 1Z2.

time for different control groups was between 9 and 12 days. Either the day of treatment (radiation therapy) or the first day of treatment (chemotherapy) was defined as Day 0, with prior and subsequent measurements of tumor volumes plotted as negative or positive times. The initial average volume of the treatment groups was chosen as the Day-0 point on the control curve. Wide variations were observed for times between implantation and the emergence of tumors to a volume of 200–400 mm^3.

Further analyses of the radiotherapy data were performed to establish the approximate $\log_{10}$ cell kill for a more meaningful interpretation of these results. The following formula was used (3):

$$\log_{10} \text{ tumor cell kill/dose} = \frac{T - C}{(3.33)\ (Td)\ (\text{No. of doses})},$$

where T = time (days) for median tumor size in treated group to reach 1000 mm^3 volume; C = time (days) for median tumor size in control group to reach 1000 mm^3 volume; Td = tumor volume doubling time; and 3.33 = No. of tumor cell doublings/1 log.

Because the initial treatment volumes in the chemotherapeutic groups were slightly different, growth delays were calculated at a specific time after the treatment. Growth delays were determined at three different points on each tumor curve, representing the volumes at 4, 6, and 8 weeks after therapy and their delay after normalization of the curve with the control curve. Statistical comparisons of the treatment and control curves were performed by analyzing the growth delay utilizing the Student's t test.

Radiotherapy Procedures

R3327H tumors in the flanks of anesthetized rats were irradiated at a volume of 200–400 mm^3 with a Picker ^{137}Ce therapy unit. Single-dose fractions of 1000, 2000, and 2500 rad were applied. The radiation dose to each tumor was administered through two different ports to facilitate homogeneous dose distribution throughout the tumor. A 2-cm cone field was utilized with the tumor pulled away from the body wall, resulting in minimal scatter and low total-body irradiation. In concurrent experiments to study the effect of the hypoxic cell radiosensitizer misonidazole (0.5 mg/g), the drug was administered ip approximately 30 minutes prior to exposure of the tumors to 1000 and 1500 rad.

Chemotherapy Treatments

The drugs tested in these preliminary studies were of interest because they were already in clinical use for genitourinary malignancies or were being tested in phase II trials. Dosages were calculated on the basis of body surface area rather than body weight. Appropriate doses were determined using experience with human drug protocols as well as guidelines in product monographs. Where necessary, an approximate LD50 was determined by using the method of Freireich et al (4).

Misonidazole was administered at a dose level that was known to produce high serum and tissue concentrations of drug and was nontoxic in single doses to other strains of rats.

Chemotherapeutic drugs were administered ip using the following schedules:

(*a*) 2 mg/m^2 of vincristine 1 day/week for 3 weeks and 20 mg/m^2 of cisplatin on 3 alternate days within 1 week;
(*b*) 30 mg/kg/day of estramustine phosphate 5 days/week for 2 weeks;
(*c*) 75 mg/m^2 of teniposide on 3 alternate days for 1 week and 150 mg/m^2 of etoposide on 3 alternate days/week for 2 weeks; and
(*d*) an initial dose of 0.2 mg/g of misonidazole and 0.1 mg/g at 2 and 4 hours on 3 alternate days for 1 week.

All treatment groups included a minimum of ten tumors. Control groups were treated with ip injections of normal saline.

RESULTS

Control growth curves using a minimum of ten tumors consistently showed a doubling time of 9–12 days. Variation in tumor size is indicated by error bars in figure 1. All treatment curves are standardized at the time of initial treatment, using one control curve.

A dose of 1000 rad was sufficient to produce a significant growth delay of the R3327H tumor. This represented a log kill of approximately 1.5, with 2000 rad producing a kill of 3.4 (table 1). The effect of radiation on tumor growth appeared to be dose-dependent, with 2500 rad producing complete tumor growth arrest for

TABLE 1.—Radiotherapy*

Therapy	Time to tumor volume of 1000 mm^3 (days)	$\log_{10}$ cell kill (Td = 9 days)
Control	16	—
1000 rad	62	1.5
2000 rad	117	3.4
2500 rad	$\geq$ 154	$\geq$ 4.6
Misonidazole + 1000 rad	112	3.2
Misonidazole + 1500 rad	$\geq$ 168	$\geq$ 5.1

*Effects of radiotherapy are expressed in terms of $\log_{10}$ cell kill utilizing formula $\frac{T - C}{3.33\ (Td)\ (\text{No. of doses})}$. Assuming regrowth of tumor at 126 days in groups that had received either 2500 rad or misonidazole + 1500 rad, minimum log cell kill can be calculated.

4–6 months (fig 1). Assuming regrowth of the tumors at 126 days, the minimum log kill is 4.6. Tumors treated with 2500 rad showed no significant shrinkage after irradiation despite the successful arrest of total tumor growth. A histologic section of an R3327H tumor in which growth had been arrested by 2500 rad of ionizing radiation for 4 months is shown in figure 2. Zones of histologically viable adenocarcinoma are evident.

Addition of misonidazole before the radiation treatment enhanced this radiation response (fig 3). Misonidazole (0.5 mg/g) had a significant effect on the response of this tumor to radiation at a dose of 1000 rad (log kill 3.2), and the sensitized response to 1500 rad was approximately equivalent to that observed with 2500 rad alone, with a minimum log kill of 5.1 (table 1).

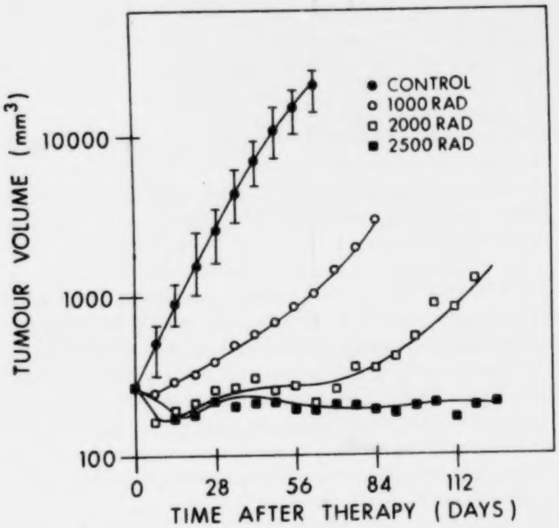

FIGURE 1.—R3327H tumor growth is inhibited with relatively small single doses of radiation. No tumor exhibited regrowth after 2500 rad; however, small palpable tumors remained. Error bars on control curve indicate both variability of tumor size and measuring error.

The chemotherapeutic drug regimens chosen resulted in moderate drug toxicity (mainly weight loss) (table 2). The drug teniposide was excessively toxic in the protocol tested and resulted in a low number of surviving animals that did not permit analysis of tumor growth with significance. The data shown in figure 4 indicate that significant growth delays ($P < 0.005$) were obtained with cisplatin, vincristine, and etoposide (table 3). The chemotherapeutic regimen with cisplatin had the greatest effect on tumor growth of the drugs tested, with an average growth delay of 4.5 weeks at 4–8 weeks after therapy. The amount of estramustine phosphate administered was relatively toxic, and the tumor growth delay that was observed (table 3) might correlate with the nutritional status of the animal, as evidenced by the severe weight loss (approximately 27%) induced by this drug (fig 5).

As a control to the irradiation studies using the hypoxic cell sensitizer misonidazole, a group of animals treated with drug alone were observed. The data of figure 6 indicate that multiple doses of the hypoxic radiosensitizer misonidazole that were sufficient to achieve adequate serum levels for several hours failed to affect the growth of the R3327H tumor in these rats.

DISCUSSION

External-beam radiotherapy is an accepted treatment modality for adenocarcinoma of the prostate in man (5,6). Fractionated-dose irradiation protocols with a total dose of up to 6000–7200 rad can result in a significant number of cures. Using the Ellis formula for nominal standard dose (NSD) (7), such clinical courses of radiotherapy produce an NSD of 1600–2000 ret. The NSD is the single-dose equivalent in rad required to produce the biologic response of the fractionated-dose irradiation protocol. Although direct extrapolation of human NSD values to experimental animal models cannot be made, the radiation doses utilized in these experiments are in a similar range. The data of figure 1 indicate that tumor volume increase is effectively controlled (for at least 6 months) by 2500 rad of radiation.

TABLE 2.—Effects of chemotherapy*

Drug	No. of— Animals	Deaths	Tumors analyzed	Mean initial tumor volume (mm^3 ± SD)	Maximum mean % weight loss
Control	13	0	15	—	—
Vincristine	7	0	10	389 ± 126	7.8
Cisplatin	12	4	11	273 ± 89	18.5
Etoposide	9	0	25	303 ± 198	7.9
Teniposide	8	4	0	309 ± 182	22.3
Estramustine phosphate	7	1	14	348 ± 280	27.7
Misonidazole	8	0	14	183 ± 65	4.0

*Only teniposide proved to be excessively toxic in terms of animal deaths. Both cisplatin and estramustine phosphate resulted in substantial weight loss.

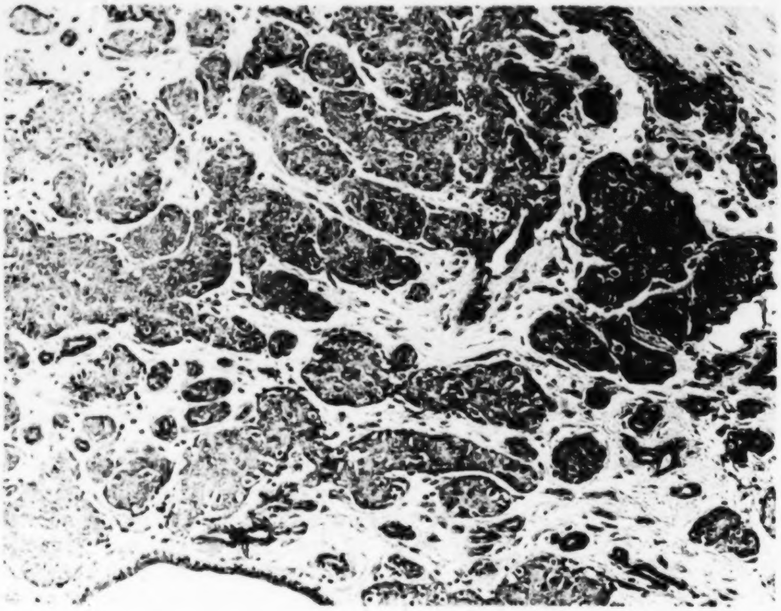

FIGURE 2.—H + E section (× 25) of an R3327H tumor 4 mos following 2500 rad. Histologic areas of apparently viable carcinoma are evident.

Single doses of 1000–2000 rad have a significant effect on tumor progression. Although increases in tumor volumes were effectively controlled with 2500-rad single doses, the tumor size was stable for several months after irradiation. Additional long-term experiments are now under way to determine the length of time this effect continues. A similar response of the R3327G tumor to ionizing radiation has been observed by Camuzzi et al (8). The persistence of palpable tumors may be related to the architecture of the tumor. R3327H is a moderately well-differentiated adenocarcinoma with a large amount of stromal tissue containing collagen that may retard tumor regression.

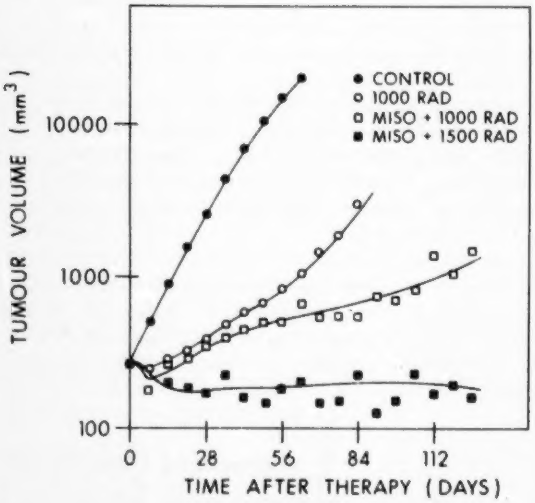

FIGURE 3.—Addition of the radiosensitizer misonidazole (0.5 mg/g) given 30 mins prior to treatment enhanced the effect of radiotherapy. Misonidazole (miso) + 1500 rad produced complete growth inhibition.

The finding that viable carcinoma is present in the nongrowing R3327H tumors for several months after treatment with 2500 rad of radiation appears to be analogous to the situation in man. The incidence of positive prostatic biopsies 1 year following attempted curative irradiation of human prostatic carcinoma is appreciable in most reports (9). Whether or not these histologically viable carcinoma cells observed in sections of irradiated R3327H tumor have proliferative and/or clonogenic potential is under current investigation. We have cultured, in vitro, an anaplastic cell line from the R3327AT tumor (courtesy of Dr. D. Coffey, Johns Hopkins University, Baltimore). Attempts will be made to culture in vitro viable cells from the hormonally responsive R3327H tumor so that cell proliferative capacity can be assayed directly.

The preliminary experiments performed with the hypoxic cell radiosensitizer misonidazole suggest that this rat tumor contains viable hypoxic cells that can play a

TABLE 3.—Tumor growth delays following chemotherapy*

Drug	Time after therapy (wks)	Growth delay (wks ± SD)	Average growth delay (wks)	P value at 8 wks
Vincristine	4	1.6 ± 0.9	2.3	< 0.005
	6	2.2 ± 1.3		
	8	3.0 ± 1.5		
Etoposide	4	1.3 ± 1.0	2.0	< 0.005
	6	2.1 ± 1.5		
	8	2.5 ± 1.9		
Cisplatin	4	3.3 ± 1.3	4.5	< 0.005
	6	4.6 ± 1.3		
	8	5.7 ± 1.9		
Estramustine phosphate	4	1.2 ± 1.4	2.7	< 0.005
	6	2.6 ± 1.9		
	8	4.2 ± 2.4		
Misonidazole	4	0.2 ± 0.9	—	—
	6	0.2 ± 1.3		
	8	0.4 ± 1.5		

*Cisplatin resulted in longest average growth delay over 4–8 wks after therapy. At 8 wks all growth delays were highly significant except with misonidazole, which showed virtually no cytotoxic effect.

role in tumor regrowth after treatment with relatively low doses of ionizing radiation. Additional studies need to be performed to precisely define sensitizer enhancement ratios as a function of drug dosage. Nevertheless, the administration of 0.5 mg/g of misonidazole 30–45 minutes prior to administration of radiation resulted in significant therapeutic enhancement. The data of figure 6 indicate that misonidazole administered alone does not have a direct cytotoxic effect on R3327H tumor cells.

Studies with cells in vitro (10) and with animal tumors in vivo (11) have indicated that hypoxia is required for this drug to have a radiosensitizing potential and/or a cytotoxic potential at the concentration of drug administered in these studies. ^{14}C-misonidazole has been used as a marker for hypoxic cells in solid tumors (12). Attempts to use ^{14}C-misonidazole to mark hypoxic cells in the R3327H tumor growing in rats have been unsuccessful to date. Examination of histologic sections of

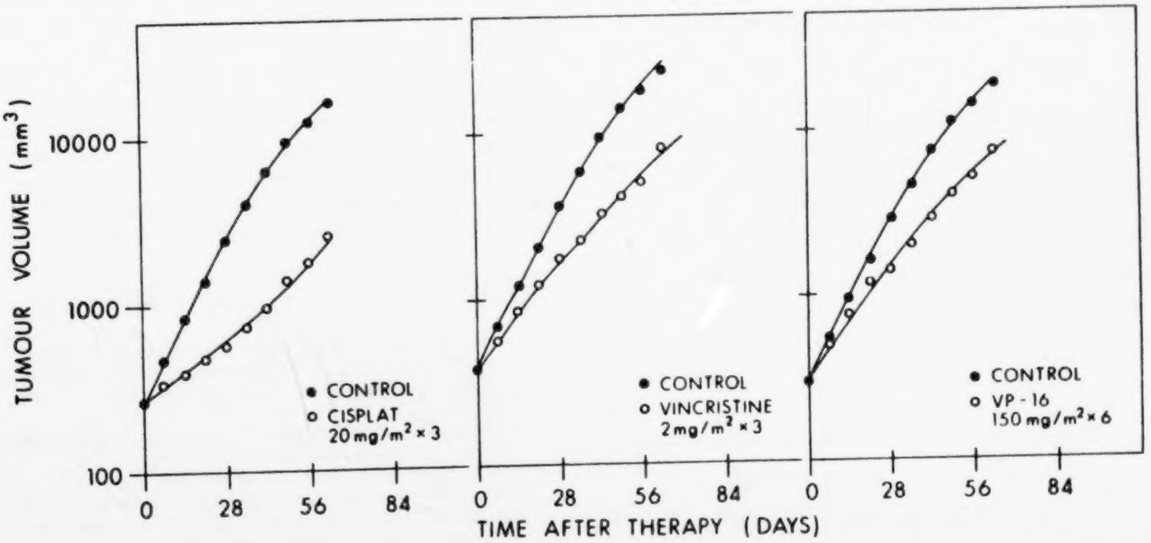

FIGURE 4.—Cisplatin (cisplat) produced a longer growth delay than either vincristine or etoposide (VP-16). All growth delays were significant at 4, 6, and 8 wks ($P < 0.005$).

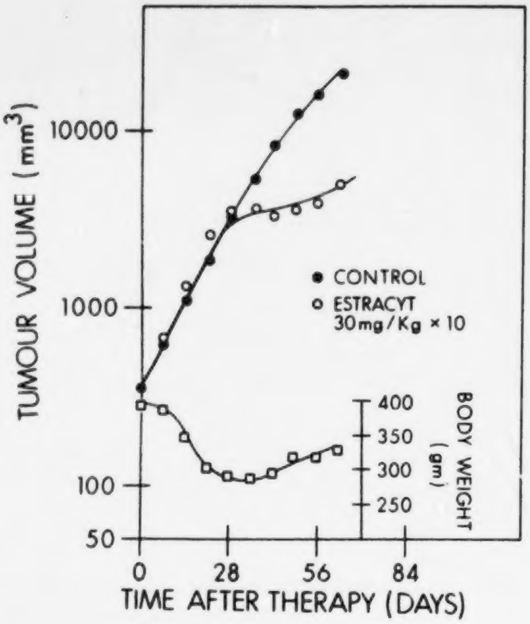

FIGURE 5.—Estramustine phosphate (Estracyt) was toxic. Significant growth delay was evident ($P < 0.005$); however, this was associated with a 27% weight loss. The role of nutrition in growth of R3327H tumor is undetermined.

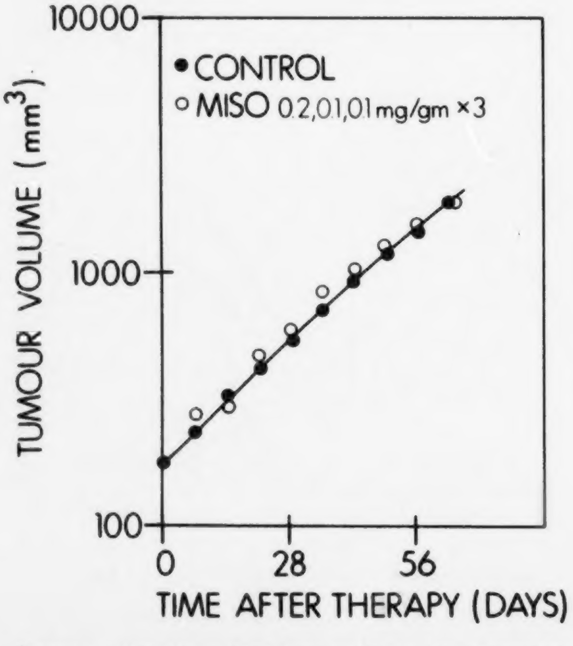

FIGURE 6.—Misonidazole (miso) given solely as a chemotherapeutic drug resulted in no growth delay.

various R3327H tumors shows good vascularization and the absence of large areas of necrosis. Additionally, the responsiveness of this tumor to relatively low doses of radiation suggests that the tumor clonogenic cells might be oxygenated. Studies are in progress to determine whether the R3327H tumors contain a component of hypoxic clonogenic cells or whether the action of misonidazole in this tumor model is unrelated to hypoxia.

The preliminary studies performed with cisplatin, vincristine, etoposide, and estramustine phosphate indicate that the rate of tumor progression can be slowed by the administration of these drugs. Complete control of this rat tumor has not been achieved in any chemotherapeutic protocol tested to date. Cisplatin had the greatest effect on slowing tumor regrowth, whereas vincristine and etoposide had a lesser effect. The drug regimen used for the administration of estramustine phosphate resulted in approximately 27% loss of body weight, and the slowing of tumor growth observed may have resulted because of the compromised nutritional status of these animals (13,14). Block et al (15,16) have shown that a variety of chemotherapeutic agents including 5-FU, cyclophosphamide, doxorubicin, cisplatin, and methotrexate have some effect on the R3327G tumor. These preliminary studies confirm a small degree of antitumor activity by cisplatin on this animal tumor. In addition, the effectiveness of vincristine and etoposide suggest that these agents could play a role in the management of human prostatic cancer.

Estramustine phosphate is presently undergoing clinical trials under the direction of the National Prostatic Cancer Project. In a detailed study with the R3327H tumor, Muntzing et al (17) reported a marked tumor regression with dosages of 100 mg/kg 3 days/week for 4 weeks. These investigators documented a 30% weight loss in the treated animals. In our studies, the tumor growth inhibition observed with this drug was transient and paralleled the severe weight loss induced in the animal, suggesting that a negative nutritional factor instead of a direct action of the drug could be responsible for the observed tumor response. Additional studies are required to distinguish between these two effects.

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Toxicity of Aziridinylbenzoquinone Administered Iv to Beagle Dogs[1,2]

Miles P. Hacker,[3,*] C. B. Hong,[3] Michael J. McKee,[3] Steven E. Unwin,[3] and Mary A. Urbanek[4]

The toxicologic effects of aziridinylbenzoquinone (AZQ) were evaluated in beagle dogs given the drug iv using several treatment schedules. AZQ was lethal to dogs at 1.25 mg/kg (25 mg/m^2) when administered as a single iv injection and at 0.31 mg/kg/day (6.2 mg/m^2/day) when administered as five daily iv injections. The lethal dose of AZQ in dogs treated with a single iv injection per week repeated for 6 consecutive weeks was 0.63 mg/kg/week (12.6 mg/m^2/week). When administered as three courses of five daily iv injections interspersed with 9 days' rest, AZQ was lethal to dogs at a dose of 0.16 mg/kg/day (3.2 mg/m^2/day). The most prominent sites of AZQ toxicity were lymphoid tissue, bone marrow, and gastrointestinal tract. Lymphoid toxicity was characterized by lymphoid depletion of spleen, thymus, lymph node, and tonsil. Bone marrow toxicity was manifested by hematologic changes (including leukopenia, thrombocytopenia, and anemia) and by hypocellularity of the bone marrow. Gastrointestinal toxicity included clinical signs (such as anorexia, emesis, diarrhea, and body weight loss) and histopathologic lesions (ie, degeneration and depletion of the intestinal crypt epithelium). Less consistent sites of AZQ toxicity were liver (characterized by increased SGPT, SGOT, and alkaline phosphatase levels and by hepatic centrilobular congestion) and kidney (manifested by elevated serum BUN). Although AZQ is highly lipid-soluble and has significant antitumor activity in intracerebrally implanted experimental tumors, no dog given AZQ developed treatment-related lesions in the brain. AZQ, a compound having significant antitumor activity in several experimental tumor systems, produced toxic effects characteristic of many anticancer drugs; the effects appeared reversible upon cessation of AZQ administration. [Cancer Treat Rep 66:1845–1851, 1982]

Doxorubicin, daunorubicin, and mitomycin are natural products with significant antitumor activity (1–4). A common chemical characteristic of these three natural products is the presence of a quinone structure. Of the several hundred purely synthetic quinones thus far screened for antitumor activity by the National Cancer Institute (NCI), Bethesda, MD, only the *p*-benzoquinone derivatives containing multiple aziridinyl groups have demonstrated antitumor activity (5).

Although doxorubicin and daunorubicin are classified as intercalating agents (6), Lin et al (7) have proposed that quinones can also undergo reduction in hypoxic cells, resulting in the production of an alkylating agent. Since the aziridinylquinones are highly lipid-soluble and possess a low degree of ionization, two requirements for CNS penetration as suggested by Rall and Zubrod (8), several of these compounds have been tested for activity against intracerebrally transplanted L1210 murine leukemia cells and against ependymoblastoma of the brain. The aziridinylbenzoquinones have significant activity in both of these tumor systems (9) and may prove to be important in the treatment of human tumors involving the CNS. The present study describes the clinical and pathologic changes in beagle dogs given aziridinylbenzoquinone (AZQ).

MATERIALS AND METHODS

Male and female beagle dogs (7–12 months of age) were supplied by Laboratory Research Enterprises (Kalamazoo, MI). The dogs were housed in individual stainless steel cages in rooms having a controlled tem-

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[1] Received Mar 4, 1982; revised June 3, 1982; accepted June 17, 1982.

[2] Supported in part by contract N01-CM-43746 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Midwest Research Institute, Kansas City, MO.

[4] Battelle Toxicology Program Office, Vienna, VA.

[*] *Reprint requests to:* Miles P. Hacker, PhD, Department of Pharmacology, Vermont Regional Cancer Center, University of Vermont, College of Medicine, Burlington, VT 05405.

perature (21°C ± 2°C), relative humidity (50% ± 5%), and photoperiod (0600–1800 hours, lighted period). Each dog was given 300 g of dog chow once daily for a 1-hour feeding period; water was provided ad libitum.

Prior to treatment, the dogs were bled at least three times at 3–4-day intervals to determine pretreatment hematologic and clinical chemistry values. Hematologic tests included the hematocrit by microtube centrifugation, hemoglobin by the cyanmethemoglobin procedure, erythrocyte and platelet counts using a Coulter counter, reticulocytes by methylene blue stain (reported as percent of erythrocytes), and differential leukocyte count by Wright's stain. Clinical chemistry parameters included BUN by the urease method; fasting blood glucose by the hexokinase method; alkaline phosphatase (AP) by the hydrolysis of *p*-nitrophenylphosphate; SGOT and SGPT by the enzymatic oxidation of nicotinamide adenine dinucleotide; creatinine by the reaction with sodium picrate; total bilirubin by the reaction with *p*-benzene diazonium sulfonate; bromsulphalein (BSP) retention by spectrophotometry; and the electrolytes Na^+, K^+, Ca^{++}, and Mg^{++} by atomic absorption and Cl^- by coulometry. For dogs given a single iv injection of AZQ, urine samples were obtained when blood samples were taken. Urine samples were tested for protein, ketones, glucose, and pH with Labstix reagent strips (Ames Co) and examined microscopically for the presence of particulate material.

A necropsy was performed on all dogs, and the following tissues were fixed in 10% neutral buffered Formalin for histopathologic examination: eye, lip, tongue, salivary gland, lymph node (mandibular, regional, bronchial, and mesenteric), tonsil, thymus, thyroid and parathyroid, trachea, esophagus, brain (anterior commissure, optic chiasm, hippocampus, cerebellum, and medulla), pituitary, middle ear, skin, mammary gland, heart, lung, spleen, adrenal, liver, gall bladder, stomach, small intestine (duodenum, jejunum, and ileum), cecum, colon, abdominal aorta, kidney, ureter, urinary bladder, gonad, prostate, uterus, rib with bone marrow, skeletal muscle, and diaphragm.

AZQ (fig 1) was supplied by the Laboratory of Toxicology, NCI, as a clinical formulation and was stored in the dark at 4°C. The 10 mg of AZQ per vial was dissolved in 0.5 ml of sterile dimethylacetamide and then diluted to a final volume of 10 ml with sterile phosphate buffer (0.01 M; pH 6.5) immediately before injection into the saphenous or cephalic vein of the dogs.

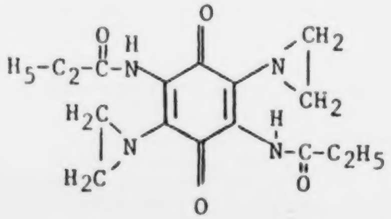

FIGURE 1.—Structure of AZQ.

The protocol used for the studies described in this report was similar to that detailed elsewhere by Prieur et al (10). Basically, dogs were given a single iv injection of AZQ (study 1), five daily iv injections (study 2), three courses of five daily iv injections interspersed with 9 days' rest (study 4), one iv injection per week for 6 consecutive weeks (study 5c), or iv injections for 10 consecutive days (study 5d). Two animals (one male and one female) were treated at each dose level tested.

For studies 1, 2, and 5d, several levels of toxicity were defined according to the protocols described by Prieur et al (10) as modified by the Laboratory of Toxicology, NCI. The defined doses were as follows: *highest nontoxic dose (HNTD)*—the highest dose tested to cause no treatment-related toxic effects; *toxic dose low (TDL)*—the lowest dose tested to cause treatment-related changes in hematologic, clinical, or morphologic parameters, but doubling this dose caused no lethality; *toxic dose high (TDH)*—dose causing treatment-related changes in hematologic, clinical, or morphologic parameters, and doubling this dose resulted in lethality; *lethal dose (LD)*—lowest dose tested to cause treatment-related deaths; and *supralethal dose (SLD)*—twice the lethal dose.

RESULTS

Single-Dose Study

A total of 12 dogs (six males and six females) were treated with a single iv injection of AZQ. One animal from each dose pair was killed on Day 8 and its partner killed on Day 46. When AZQ was administered at either the SLD or LD, the animals were killed when they became moribund. The SLD, LD, TDH, TDL, and HNTD for this treatment regimen are summarized in table 1, while the toxic effects of AZQ administration are summarized in table 2. Treatment-related deaths occurred as late as Day 10 in this study. The acute effects of AZQ administration included gastrointestinal toxicity, pyrexia, and lethargy. Doses of AZQ less than the LD caused less severe and more sporadic signs of toxicity. No treatment-related changes were detected in the urine of any dog administered a single iv injection of AZQ.

AZQ caused pancytopenia, which was most prominent during the first 8 days of the study, in dogs treated with ≥ 0.63 mg/kg (≥ 12.6 mg/m^2). Changes in clinical chemistry values of AZQ-treated dogs were limited to elevated AP and SGPT in dogs given either the LD or TDH of AZQ.

Histopathologic examination of tissues taken from dogs treated with a single iv injection of AZQ revealed

TABLE 1.—Defined doses for AZQ administered iv to dogs

Study No.	Regimen	Defined dose	mg/kg/injection	mg/m^2/injection
1	Single iv injection	SLD	2.50	50
		LD	1.25	25
		TDH	0.63	12.6
		TDL	0.16	3.2
		HNTD	0.08	1.6
2	5 daily iv injections	SLD	0.63	12.6
		LD	0.31	6.2
		TDH	0.16	3.2
		TDL	0.08	1.6
		HNTD	0.04	0.8
5d	10 daily iv injections	LD	0.31	6.2
		TDH	0.16	3.2
		TDL	0.04	0.8
		HNTD	0.02	0.4

TABLE 2.—Toxic effects of AZQ administered as a single iv injection to dogs

Dose:	SLD		LD		TDH				TDL		HNTD	
mg/kg:	2.5		1.25		0.63		0.31		0.16		0.08	
mg/m^2:	50		25		12.6		6.2		3.2		1.6	
Dog No.:	*481M*	*484F*	*485M*	*480F*	*483M*	*482F*	*487M*	*486F*	*489M*	*488F*	*509M*	*510F*
Day died (D) or killed (K)	6 (D)	5 (D)	8 (K)	10 (D)	8 (K)	46 (K)	8 (K)	46 (K)	46 (K)	8 (K)	46 (K)	8 (K)
Clinical signs												
Decreased food intake	X*	X	X	X		X		X			X	
Body weight loss	X	X	X	X								
Emesis	X	X	X	X								
Diarrhea	X		X			X	X					
Mucus and/or blood in feces		X	X	X			X	X	X	X		
Pyrexia			X	X								
Blood values												
Decreased hematocrit			25† (8)‡	30 (8)	15 (8)	20 (22)						
Decreased hemoglobin				20 (8)								
Decreased erythrocytes		20 (4)	30 (8)	33 (8)	15 (8)	15 (22)		20 (4)				
Decreased leukocytes	40 (4)	88 (4)	90 (8)	80 (8)	70 (8)	80 (8)	40 (8)		40 (8)	25 (4)		
Decreased reticulocytes	95 (4)	95 (4)	95 (8)	95 (8)	95 (8)	95 (8)	95 (4)	95 (4)	90 (4)			
Decreased platelets		40 (4)	80 (8)	33 (8)		33 (8)		27 (4)				
Increased AP			400 (8)	270 (8)		340 (8)						
Increased SGPT			1350 (8)									
Tissue lesions												
Lymphoid depletion												
Spleen	4§	4	3	4								
Thymus	NT	NT	3	3	1							
Lymph node	2	4	2	3	1							
Tonsil	3	4	3	3		1						
Hypocellular bone marrow	4	4	3	3								
Degeneration and/or depletion of small intestine epithelium	2	4	1	1	1							
Centrilobular congestion	1			1								

*X = clinical sign of toxicity was observed.

†% change from the average baseline values of 3 blood samples taken prior to drug administration.

‡Day in study when change was most pronounced.

§Severity of tissue lesion: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; NT = tissue not taken.

lymphoid depletion of the lymph nodes, hypocellularity of the bone marrow, depletion or degeneration of intestinal epithelium, and centrilobular congestion of the liver. Except for a mild lymphoid depletion in dogs given 0.31 mg/kg (6.2 mg/m^2), these lesions were detected only at the LD or SLD of AZQ. No significant histopathologic lesions were observed in tissues taken from dogs killed on Day 46 of the study.

Five Daily Dose Study

A total of 12 dogs (six male and six female) were treated with five daily iv injections of either AZQ or the drug solvent. One animal of each dose pair was killed on Day 6, and the remaining dog was killed on Day 51–53. When AZQ was administered at either the SLD or LD, however, animals were killed either on Day 6 or when the animal became moribund. The defined doses are summarized in table 1, and the toxic effects of AZQ are listed in table 3. Spontaneous deaths occurred as late as Day 11 (6 days after the final AZQ injection) with this treatment regimen. The toxic signs were quite similar to those described for dogs given a single iv injection of AZQ and were limited to dogs treated with either the SLD or the LD of AZQ. The clinical signs of toxicity became evident by Day 4 and increased in severity until the ultimate death of the animal.

Pancytopenia was noted during the first 2 weeks of the study in dogs treated with 0.08 mg/kg/day (1.6 mg/m^2/day). This hematologic alteration was evidently reversible, since all values had returned to near baseline levels by Day 23 of the study.

The major treatment-related tissue lesions were lymphoid depletion of spleen, thymus, lymph node, and tonsil; hypocellularity of the bone marrow; depletion or degeneration of the intestinal crypt epithelium; intestinal hemorrhage; and hepatic centrilobular congestion. These lesions were limited primarily to dogs given the SLD or LD of AZQ.

Multiple-Dose Studies With Periods of Rest Between Treatment

Six dogs were treated with three courses of five daily iv injections of AZQ interspersed with a 9-day rest period between each course of AZQ treatment (study 4).

TABLE 3.—Toxic effects of AZQ administered as 5 daily iv injections

Dose:	SLD		LD		TDH		TDL		HNTD			
mg/kg/day:	0.63		0.31		0.16		0.08		0.04		0.0	
mg/m²/day:	12.6		6.2		3.2		1.6		0.80		0.0	
Dog No.:	*495M*	*496F*	*493M*	*494F*	*491M*	*492F*	*507M*	*508F*	*525M*	*524F*	*505M*	*506F*
Day died (D) or Killed (K)	6 (D)	11 (K)	11 (D)	6 (K)	6 (K)	52 (K)	6 (K)	53 (K)	6 (K)	51 (K)	6 (K)	53 (K)
Clinical signs												
Decreased food intake	X	X	X	X								
Body weight loss	X	X	X									
Emesis	X		X									
Diarrhea	X											
Blood and/or mucus in feces	X	X	X		X							
Pyrexia	X	X	X									
Lethargy	X											
Blood values												
Decreased hematocrit		30 (9)	20 (9)	25 (6)				15 (9)				
Decreased hemoglobin		27 (9)	15 (9)	18 (6)								
Decreased erythrocytes		28 (9)	25 (9)	18 (6)								
Decreased leukocytes		88 (9)	90 (9)	48 (6)	38 (4)	70 (9)	40 (4)	45 (9)				
Decreased reticulocytes	88 (4)	90 (9)	95 (9)	90 (6)	95 (6)	80 (9)		85 (9)				
Decreased platelets		36 (9)	25 (2)			50(16)						
Increased AP			550 (9)									
Tissue lesions												
Lymphoid depletion												
Spleen	4	3	3	1	1							
Thymus	4	3	3	1								
Lymph node	4	4	3	3	1							
Tonsil	4	NT	3	1	1							
Hypocellular bone marrow	4	4	4	2	2							
Degeneration and/or depletion of intestinal epithelium	4	3	1	1			1					
Centrilobular congestion		1	1									

TABLE 4.—Toxic effects of AZQ administered as 3 courses of 5 daily iv injections with 9 days' rest (study 4) or as weekly iv injections (study 5c)

Study No.:	4		4		4		5c		5c	
Dose: mg/kg/injection:	0.16		0.08		0.04		0.63		0.16	
mg/m²/injection:	3.2		1.6		0/80		12.6		3.2	
Dog No.:	*513M*	*514F*	*511M*	*512F*	*569M*	*568F*	*503M*	*504F*	*499M*	*500F*
Day died (D) or killed (K)	16 (D)	15 (D)	34 (K)	79 (K)	80 (K)	34 (K)	9 (K)	12 (K)	37 (K)	81 (K)
Clinical signs										
Decreased food intake	X	X					X	X	X	X
Body weight loss	X	X		X			X	X		X
Emesis	X						X	X		X
Diarrhea	X	X		X			X	X		
Blood in feces	X	X		X	X		X	X		
Pyrexia	X	X					X	X		
Blood values										
Decreased hematocrit	20 (15)	25 (11)		15 (15)		29 (16)		28 (9)		
Decreased hemoglobin	14 (16)	25 (11)		15 (15)		27 (16)		15 (9)		
Decreased erythrocytes	25 (16)	30 (11)		15 (15)		30 (16)		28 (9)		
Decreased leukocytes	90 (11)	88 (11)	40 (30)	45 (30)		46 (16)	90 (9)	90 (9)	60 (18)	40 (18)
Decreased reticulocytes	95 (9)	95 (11)	65 (23)	57 (25)	95 (16)	95 (4)	80 (4)	90 (11)	80 (18)	80 (18)
Decreased platelets	77 (15)	55 (11)	50 (29)	33 (30)	75 (16)		67 (9)	70 (8)	20 (23)	
Decreased glucose							30 (9)	30 (11)		
Increased BUN	400 (16)						475 (9)	200 (11)		
Increased BSP retention	520 (15)									
Increased AP	520 (16)	60 (11)					350 (9)	375 (11)		
Increased SGPT									390 (16)	
Tissue lesions										
Lymphoid depletion										
Spleen	2	2					2	2		
Thymus	NT	4					2	4		
Lymph node	3	2					2	2		
Tonsil	3	3					2	2		
Hypocellular bone marrow	2	4					3	3		
Degeneration and/or depletion of intestinal epithelium	2	2	1	2	1	1	3	2		
Centrilobular congestion	2	1						1		

The dose levels used in this study were the TDH, TDL, and HNTD as defined for study 2. Only the TDH dose (0.16 mg/kg/day; 3.2 mg/m²/day) caused significant signs of toxicity, first observed on Day 11 and increasing in severity until the eventual death of the animal (table 4). Pancytopenia was observed in both dogs treated with 0.16 mg/kg/day, with less severe and more sporadic changes in dogs given lesser doses of AZQ. Elevated BUN, BSP retention, and AP were noted in one dog administered 0.16 mg AZQ/kg/day. No other clinical chemistry changes were detected in dogs treated with this dosing regimen. As with the other treatment schedules, lymphoid depletion, bone marrow hypocellularity, intestinal degeneration, and hepatic centrilobular congestion were characteristic of AZQ-induced tissue lesions. These histopathologic alterations were prominent only in dogs given 0.16 mg/kg/day. Although elevated BUN levels were sporadically detected in animals administered AZQ, no treatment-related lesions were observed in the kidneys of any animal treated with AZQ.

Four dogs were treated with a single iv injection of either 0.63 or 0.16 mg/kg/week (12.6 or 3.2 mg/m²/week) for up to 6 consecutive weeks (study 5c). The doses chosen for this study were the TDH and TDL as defined for study 1. Both dogs given 0.63 mg/kg/week died after receiving two courses of AZQ treatment. The toxic signs, similar to those described in previous studies, were first evident on Day 8 and became progressively worse until the eventual death of the animal (table 4). Hematologic and clinical chemistry changes were detected primarily in dogs treated with 0.63 mg AZQ/kg/week and included pancytopenia, elevated levels of BUN and AP, and decreased blood glucose. The histopathologic lesions noted in the tissues of those dogs were typical of the lesions described previously for AZQ.

When administered as ten daily iv injections (study 5d), the defined doses of TDL and HNTD for AZQ were one-half the amount (in mg/kg/day) of those given as five daily injections (table 1). As shown in table 5, the toxic signs were similar to those described for other treatment schedules and were observed in dogs given either 0.31 or 0.16 mg of AZQ/kg/day (6.2 or 3.2 mg/

TABLE 5.—Toxic effects of AZQ administered as 10 daily iv injections

Dose: mg/kg/day:	0.31		0.16		0.08		0.04		0.02	
mg/m^2/day:	6.2		3.2		1.6		0.8		0.4	
Dog No.:	*521M*	*516F*	*517M*	*520F*	*519M*	*518F*	*523M*	*522F*	*571M*	*570F*
Day died (D) or killed (K)	11 (K)	12 (D)	11 (K)	58 (K)	11 (K)	58 (K)	11 (K)	57 (K)	11 (K)	58 (K)
Clinical signs										
Decreased food intake	X	X	X	X						X
Body weight loss	X	X	X	X	X	X	X	X		
Emesis	X									
Diarrhea				X						
Blood and/or mucus in feces				X						
Pyrexia	X	X								
Blood values										
Decreased hematocrit	15 (11)	15 (11)		18 (11)						
Decreased hemoglobin	30 (11)			20 (11)						
Decreased erythrocytes	15 (11)	23 (11)		20 (11)						
Decreased leukocytes	95 (11)	97 (11)	76 (11)	70 (11)	40 (11)	50 (11)	23 (6)	40 (11)		
Decreased reticulocytes	100 (11)	95 (11)	95 (11)	95 (11)	60 (7)	95 (11)				
Decreased platelets	99 (11)	92 (11)	18 (11)	20 (11)	45 (4)					
Increased BUN	160 (11)									
Increased BSP retention	500 (11)									
Increased AP	660 (11)	700 (11)		160 (18)						
Increased SGOT	35 (11)	30 (11)		40 (2)						
Increased SGPT		40 (11)		30 (11)						
Tissue lesions										
Lymphoid depletion										
Spleen	3	3	1							
Thymus	3	3	2		1					
Lymph node	3	3	1		1					
Tonsil	4	3	1							
Hypocellular bone marrow	4	4	4							
Degeneration and/or depletion of intestinal epithelium	2	2	1		1		1			
Centrilobular congestion	1	1								

m^2/day). Clinical signs of toxicity were first noted on Day 10 in dogs receiving 0.31 mg of AZQ/kg/day, and by Day 12, both dogs were dead. Hematologic, clinical chemistry, and histopathologic changes associated with this dosing regimen were similar to those previously described for all other regimens.

DISCUSSION

The defined doses of LD, TDH, TDL, and HNTD for AZQ given iv to dogs following various dose regimens are summarized in table 1. As indicated by studies 1 and 2, AZQ was approximately equitoxic whether given as a single iv injection or as five daily iv injections, when total dose administered was compared. The toxicity of AZQ was apparently increased, however, by two of the treatment schedules tested. The TDH of study 2 was lethal to dogs when a 9-day rest period was interposed between each series of five daily doses (study 4). Similarly, the TDH of study 1 was lethal to dogs when given as single doses at weekly intervals (study 5c). This increased toxicity by the divided dosing regimen was not totally unexpected, however, since the most significant changes brought about by AZQ treatment were somewhat delayed in onset, first appearing 6–15 days after the initial dose of AZQ. Thus, if a second course of AZQ treatment were begun during this time, it is conceivable that the toxicity of AZQ would be increased.

Regardless of the dosing regimen, the most prominent toxic effects produced by iv treatment with AZQ occurred in the lymphoid tissue, bone marrow, and intestine. More sporadic and less severe toxic effects were also detected in the liver and kidney of the dogs. The incidence and severity of these lesions were directly related to the dose of AZQ given and were limited primarily to animals given the TDH or greater dose. Regardless of the dosing regimen, the toxic effects of AZQ were apparently reversible upon cessation of drug administration.

Lymphotoxicity was manifested primarily as lymphoid depletion of the lymph node, spleen, tonsil, and thymus. Other treatment-related lymphoid changes included hemorrhage in the lymph nodes and ulceration of the tonsil.

The toxic effects of AZQ on the bone marrow were characterized by alterations in the hematologic values and by hypocellularity. Severe leukopenia, thrombocytopenia, and reticulocytopenia were observed in dogs treated with the LD of AZQ. Less severe, but still quite prominent, decreases in leukocyte and reticulocyte counts were also noted at the TDH dose level. The bone marrow was mildly to severely hypocellular in all animals given the TDH or greater dose of AZQ and necropsied prior to the last scheduled necropsy date for a given study. Since the myeloid/erythroid ratios were not altered in any of these animals, AZQ apparently had no specific effect on either the myelogenous or erythrocytic series of cells.

The gastrointestinal toxicity of AZQ was indicated by signs such as decreased food intake; emesis; diarrhea; and blood, bile, and/or mucus in the feces. Histopathologic examination of the gastrointestinal tract revealed depletion and degeneration of crypt epithelium.

Results of liver function tests, including elevated AP, SGOT, SGPT, and/or BSP retention time, indicated that hepatic damage had occurred following AZQ administration. These changes were observed most often during the first 10 days following administration of AZQ at the TDH or greater dose level. Histopathologic examination revealed centrilobular congestion in the livers of several dogs. Although renal toxicity was not detected by histopathology, there was clinical chemistry evidence of renal damage, in that several animals given the TDH or greater dose of AZQ developed elevated serum BUN.

In summary, AZQ produced significant toxic effects to the lymphoid tissues, bone marrow, intestine, kidney, and liver. The toxic effects of AZQ were similar for all treatment schedules tested. The changes occurred primarily in animals given the TDH or greater dose of AZQ and were reversible in those dogs that survived the entire recovery period. In addition, there appeared to be a rather prolonged latency period of up to 10 days between the initiation of AZQ treatment and the development of severe toxic changes, which may explain the increased AZQ toxicity observed when the drug was given on an intermittent basis. It is of interest that AZQ, which has the chemical characteristics required for good CNS penetration, caused no detectable changes in the CNS at less than lethal doses of the drug.

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3-Deazaguanine, a Candidate Drug for the Chemotherapy of Breast Carcinomas?[1,2]

Tasneem A. Khwaja[3,4,*]

3-Deazaguanine (3-DG) is a new purine antagonist that is active against slow- and rapid-growing solid experimental tumors, especially those that are models for human breast carcinomas. These tumors include mammary adenocarcinomas 13762, R3230AC, and C3H/16C. 3-DG also showed positive activity against leukemias L1210 and L1210/araC, adenocarcinoma 755, and EMT-6 mammary adenocarcinoma. On the basis of its activity against these tumors, 3-DG is to undergo clinical trials. [Cancer Treat Rep 66:1853–1858, 1982]

3-Deazaguanine (3-DG) is a structural modification of guanine in which nitrogen in the 3-position of the purine ring has been replaced with a carbon atom and a hydrogen atom (fig 1). The synthesis of 3-DG was accomplished by Cook et al (1), who also described its potent in vitro antiviral activity against both DNA and RNA viruses (2). The antitumor activity and biochemical pharmacology of 3-DG have been briefly reported (3–6). In this report the details of the antitumor activity of 3-DG are presented.

MATERIALS AND METHODS

Sources of animals and tumors.—The animals were obtained through Mr. Clarence R. Reeder, Animal Genetics and Production Branch, Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD. The tumor lines were obtained from Southern Research Institute, Birmingham, AL (leukemias L1210, L1210/araC, and L1210/6MP; adenocarcinoma 755; mammary adenocarcinoma C3H/16C; colon adenocarcinoma 38; Lewis lung carcinoma; and B16 melanotic melanoma); Dr. Russel Hilf, University of Rochester, Rochester, NY (mammary adenocarcinoma R3230AC); Mason Research Institute, Worcester, MA (mammary adenocarcinoma 13762), Arthur D. Little, Inc, Boston, MA (L1210 XII/FU); and Dr. Sarah Rockwell, Yale University, New Haven, CT (EMT-6 mammary adenocarcinoma).

Maintenance of animals and tumors.—These tumors were maintained by serial transplantation in appropriate hosts (or when not in use were kept frozen in a liquid nitrogen freezer) in the Animal Tumor Research Facility of the Los Angeles County-University of Southern California Comprehensive Cancer Center. The animals in each shipment were quarantined, and the acceptable animals were kept in holding rooms until their weights were appropriate (mice, 18–22 g; rats, 70–90 g) for tumor transplantation or experimentation. Tumor transplantations were carried out in separate clean laboratories using standard protocols established by the Drug Evaluation Branch of the National Cancer Institute. Leukemia L1210 was maintained by ip inoculation of the ascites cells (1×10^5) in DBA/2 mice. Leukemia L1210/araC was maintained by weekly ip transplants of 1×10^5 cells in BDF$_1$ (C57BL/6 × DBA/2) female mice. Leukemias L1210/6MP and L1210/FU

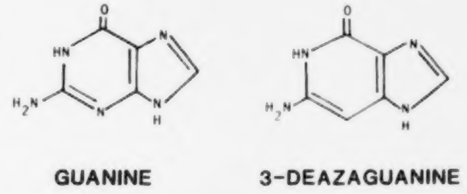

FIGURE 1.—Structures of guanine and 3-DG.

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[1] Received Mar 10, 1982; revised June 28, 1982; accepted July 12, 1982.

[2] Supported by Public Health Service grants CA-14089 and CA-25715 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Pathology, University of Southern California School of Medicine, and USC Comprehensive Cancer Center, Los Angeles.

[4] I am thankful to Ms. Janet C. Varven and Ms. Stephanie Pentecost for excellent technical assistance.

[*] *Reprint requests to:* Dr. Tasneem A. Khwaja, University of Southern California, Comprehensive Cancer Center, 1720 Zonal Ave, Los Angeles, CA 90033.

were maintained by weekly serial transplantation of 1 × 10^6 cells in DBA/2 female mice. Resistance to 6-mercaptopurine (6-MP) was maintained by five daily sc injections of 6-MP (10 mg/kg) beginning 24 hours after transplant. L1210 XII (L1210/FU) mice were treated with seven daily ip injections of 5-FU at a dose of 20 mg/kg of body weight. Adenocarcinoma 755, B16 melanoma, Lewis lung carcinoma, and colon adenocarcinoma 38 were maintained by serial tumor (2–4-mm^3 pieces) transplants (sc in left axillary region) in C57BL/6 female mice. Similarly, mammary adenocarcinomas R3230AC and 13762 were maintained in Fischer 366 female rats. EMT-6 mammary adenocarcinoma was maintained in suspension culture in Eagle's minimum essential medium containing 10% fetal calf serum; each animal ($CD2F_1$ female) received 2 × 10^6 cells sc for experimentation. Lung-passed mammary adenocarcinoma C3H/16C was maintained in C3H female mice according to the method of Corbett et al (7).

Drugs and drug preparations.—3-DG was obtained from ICN Pharmaceuticals, Inc, through the courtesy of Dr. Roland K. Robins. Other standard control drugs were obtained from the NCI Drug Evaluation Branch. For injection purposes, 3-DG was dissolved in sterile 0.2 M phosphate buffer (pH 7.5). 5-FU and 6-MP were dissolved in sterile physiologic saline (pH adjusted to 9.0 with NaOH). All injection solutions were kept at 0°C when not in use. Stability of 3-DG was periodically monitored by high-pressure liquid chromatography.

Test evaluations.—Test evaluations were carried out on the basis of the mean survival time or mean tumor weight of each test group (T) as compared to the control group

TABLE 1.—Effect of 3-DG on growth of L1210 leukemias in BDF_1 female mice

Tumor system	Treatment, ip (Days)	Mean survival (days)	Δ weight on Day 5 (g)	T/C	ILS (%)	No. of survivors at Day 30/No. of mice treated
L1210 (ip) 1 × 10^6 cells	Saline	8.20	+2.20	—	—	0/10
	3-DG					
	20 mg/kg, qd (1–9)	10.83	+2.00	1.32	32	0/6
	40 mg/kg, qd (1–9)	12.00	+0.40	1.46	46	0/6
	80 mg/kg, qd (1–9)	13.33	+1.70	1.63	63	0/6
	Cytarabine					
	40 mg/kg, qd (1–9)	17.33	−0.20	2.11	111	0/6
L1210/araC (ip) 1 × 10^5 cells	Saline	8.80	+1.30	—	—	0/10
	3-DG					
	40 mg/kg, qd (1–9)	13.20	−0.67	1.50	50	0/6
	80 mg/kg, qd (1–9)	12.20	−1.17	1.39	39	0/6
	Cytarabine					
	40 mg/kg, qd (1–8)	8.20	−0.83	0.93	−7	0/6
L1210/6MP (ip) 1 × 10^6 cells	Saline	12.63	+1.80	—	—	0/10
	3-DG					
	40 mg/kg, qd (1–9)	12.17	+1.17	0.96	−4	0/6
	80 mg/kg, qd (1–9)	10.67	+0.50	0.84	−16	0/6
	6-MP					
	35 mg/kg, qd (1–9)	12.67	+0.67	1.00	0	0/6
L1210/FU (ip) 1 × 10^6 cells	Saline	12.33	+2.82	—	—	1/10
	3-DG					
	80 mg/kg, qd (1–9)	13.80	+0.50	1.12	12	0/6
	5-FU					
	25 mg/kg, qd (1–7)	9.33	+0.33	0.76	−24	0/6
L1210 (ic)* 1 × 10^4 cells	Saline	9.75	+0.17	—	—	0/8
	3-DG					
	40 mg/kg, qd (1–9)	10.67	+0.17	1.09	9	0/6
	80 mg/kg, qd (1–9)	12.83	+0.33	1.32	32	0/6

*ic = intracerebral.

TABLE 2.—Effect of 3-DG on growth of various transplantable solid tumors

Tumor system (sc)	Host	Treatment* (Days)	Tumor weight, in mg	Δ weight on Day 5 (g)	No. of animals with palpable tumors	T/C	Tumor growth inhibition (%)
Adenocarcinoma 755	C57BL/6 mice	Saline	926 (Day 14)	+0.32	8/8	—	—
		3-DG					
		40 mg/kg, qd (1–7)	227 (Day 14)	+0.64	6/6	0.24	76
		80 mg/kg, qd (1–7)	62 (Day 14)	−2.33	2/6	0.07	93
		6-MP					
		35 mg/kg, qd (1–7)	48 (Day 14)	−0.20	2/6	0.05	95
Mammary adenocarcinoma 13762	Fischer rats	Saline	2110 (Day 16)	+8.00	12/12		
		3-DG					
		40 mg/kg, qd (1–9)	800 (Day 16)	+4.00	8/8	0.38	62
		80 mg/kg, qd (1–9)	60 (Day 16)	+4.75	6/8	0.03	97
		Estradiol valerate (sc)					
		14.4 mg/kg, qd (1–7)	2740 (Day 16)	+0.75	4/4	1.3	−30
		5-FU					
		25 mg/kg, qd (1–7)	1560 (Day 16)	−2.00	8/8	0.74	26
Mammary adenocarcinoma R3230AC	Fischer rats	Saline	4540 (Day 21)	+14.8	10/10	—	—
		3-DG					
		20 mg/kg, qd (1–9)	2530 (Day 21)	+11.3	4/4	0.56	44
		40 mg/kg, qd (1–9)	650 (Day 21)	+8.8	2/4	0.14	86
		80 mg/kg, qd (1–9)	100 (Day 21)	+4.8	1/8	0.01	99
		5-FU					
		25 mg/kg, qd (1–7)	1130 (Day 21)	+7.3	2/4	0.25	75
		Estradiol valerate (sc)					
		12.9 mg/kg, qd (1–7)	2620 (Day 21)	−1.0	2/4	0.54	46
EMT-6 mammary adenocarcinoma	$CD2F_1$ mice	Saline	414 (Day 17)	+0.41	8/8	—	—
		3-DG					
		80 mg/kg, qd (3–10)	170 (Day 17)	−0.80	7/8	0.41	59
Colon adenocarcinoma 38	BDF_1 mice	Saline	120 (Day 17)	+0.4	10/10	—	—
		3-DG					
		80 mg/kg, qd (1–9)	80 (Day 17)	−0.67	2/6	0.66	34
		5-FU					
		98 mg/kg, qd (3,10,17)	0	−0.83	0/6	0	100
Lewis lung carcinoma	C57BL/6 mice	Saline	625 (Day 12)	+0.32	8/8	—	—
		3-DG					
		40 mg/kg, qd (1–7)	608 (Day 12)	+0.72	8/8	0.97	3
		80 mg/kg, qd (1–7)	581 (Day 12)	+0.11	8/8	0.93	7
B16 melanoma	C57BL/6 mice	Saline	496 (Day 14)	+0.32	10/10	—	—
		3-DG					
		80 mg/kg, qd (1–9)	940 (Day 14)	−1.00	6/6	1.91	−91
		5-FU					
		20 mg/kg, qd (1–7)	160 (Day 14)	−1.33	6/6	0.32	68

*Unless otherwise specified, injections were given ip.

(C), which received only sterile isotonic saline, and results are expressed as the ratio of T/C obtained from each experiment. Tumor weights (mg) were estimated by measuring length (l) and width (w) of each tumor with a vernier caliper (mm) and using the formula $\frac{l \times (w)^2}{2}$. Any animal weight gain or loss during the first 5 days of each test was recorded. Mortality > 34% in treatment groups in tumor inhibition studies or death within 5 days of initial transplant in survival studies was considered to be due to drug toxicity. Weight loss (eg, > 4 g/animal in mice) was also regarded as a sign of drug toxicity, and antitumor results obtained were not considered. An increase in lifespan (ILS) > 25% in survival time assays for ascites tumors or an inhibition in tumor weight > 58% (solid tumors) was considered significant.

RESULTS

Table 1 shows that 3-DG is active against leukemias L1210 (ILS, 63%) and L1210/araC, the L1210 strain resistant to treatment with cytarabine (ILS, 50%). 3-DG was not active against L1210/6MP or L1210/FU, the L1210 strains that are resistant to 6-MP and 5-FU treatment, respectively. In solid tumor systems (table 2), 3-DG almost completely inhibited the growth of adenocarcinoma 755, mammary adenocarcinoma 13762, and mammary adenocarcinoma R3230AC, resulting in tumor weight inhibition of 93%, 97%, and 98%, respectively. 3-DG was very effective in inhibiting the growth of C3H mammary adenocarcinoma 16/C (table 3 and fig 2) but showed marginal activity against EMT-6 mammary adenocarcinoma and colon adenocarcinoma 38 (table 2). 3-DG was inactive in Lewis lung carcinoma and B16 melanoma systems (table 2). 3-DG was also tested for its activity against intracerebral implants of leukemia L1210 (1×10^4 cells/mouse) when the drug was administered ip. Our studies show (table 1) that 3-DG was active in this system (ILS, 32%).

DISCUSSION

3-DG in experimental animal leukemia models (table 1) exhibits acceptable activity against leukemia L1210 and cytarabine-resistant leukemia L1210 lacking deoxycytidine kinase. 3-DG was not active against L1210/6MP leukemia lacking hypoxanthineguanine phosphoribosyl transferase and L1210/FU lacking a pyrimidine phosphoribosyl transferase, suggesting that the drug needs to be phosphorylated to be effective (4). In solid tumor systems (tables 2 and 3), 3-DG treatments almost completely inhibited the growth of adenocarcinoma 755, mammary adenocarcinoma 13762, and mammary adenocarcinoma R3230AC. These results are extremely

TABLE 3.—Effect of 3-DG on growth of 16/C mammary adenocarcinoma in B6C3F$_1$ female mice[*]

Treatment, ip (Days)	Median survival time (days)	% ILS	Duration of remission: T – C value (days)	Activity rating[†]
Saline	48	—	—	—
3-DG				
40 mg/kg, qd (1–9)	50 (42.5)[†]	4 (31)[†]	4.0 (9.5)[†]	+ (+ +)
80 mg/kg, qd (1–9)	65.5	36	7.5	+ +
5-FU				
20 mg/kg, qd (1–9)	53	10	2.5	+
Saline	34	—	—	—
3-DG				
400 mg/kg, qd (3,7,10,14,17)	62	82	10.2	+ +
5-FU				
98 mg/kg, qd (3,7,14)	62	82	15.0	+ +
Saline	29.5	—	—	—
3-DG				
400 mg/kg, qd (3,10,17,24)	68.0	130	7.0	+ +
400 mg/kg, qd (3,7,10,14,17,21,24)	74.0	150	22.0	+ + + +
5-FU				
98 mg/kg, qd (3,10,17,24)	55.5	88	17.0	+ + +

[*] Groups of 6–8 mice were given sc transplants of 1–2-mm^3 fragments of tumor on Day 0. Treatments were started 24 hrs or 3 days after implant. T – C value represents tumor growth delay and is based on time required by treatment groups to reach 750 mg in weight minus time required by controls to reach same weight.

[†] Ref. 7.

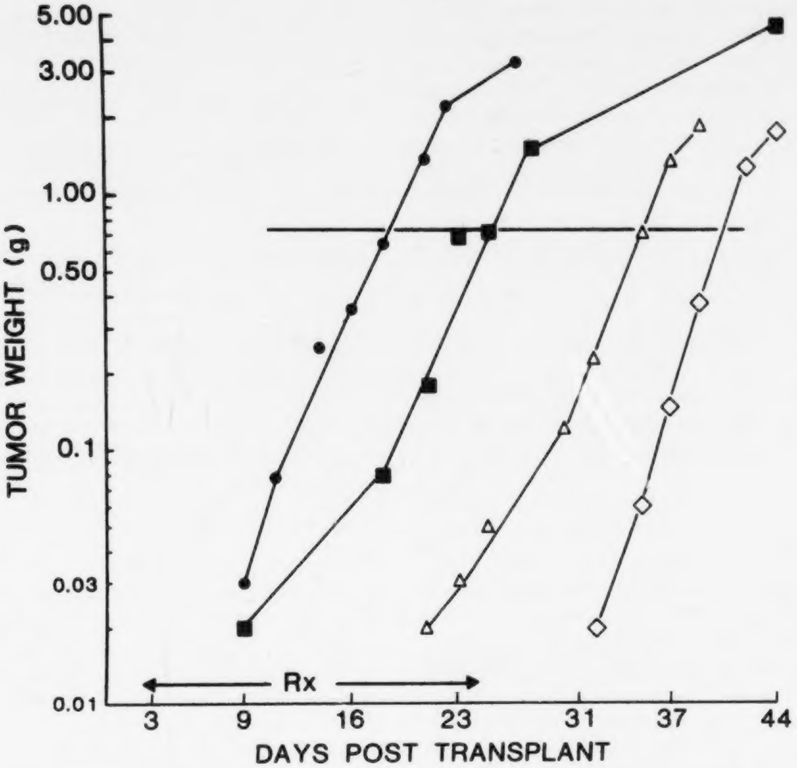

FIGURE 2.—Effect of 3-DG on growth of C3H/16C mammary adenocarcinoma in B6C3F$_1$ female mice. ● = saline controls; ■ = 3-DG, 400 mg/kg, Days 3, 10, 17, and 24; Δ = 5-FU, 98 mg/kg, Days 3, 10, 17, and 24; and ◇ = 3-DG, 400 mg/kg, Days 3, 7, 10, 14, 17, 21, and 24.

interesting in the sense that mammary adenocarcinoma R3230AC is a non-hormone-dependent, estrogen-sensitive, slow-growing tumor, and a model for postoperative breast carcinomas. Mammary adenocarcinoma 13762 is a rapidly growing non-hormone-dependent tumor that is not responsive to estrogen treatment (8). Both of these tumors are differentiated and, under hormonal stimulation, show milk-like secretions (8–10). They represent models analogous to different types of human breast cancers and differ in their responsiveness to treatment with conventional chemotherapeutic agents (8–12). 3-DG was also evaluated against C3H mouse mammary adenocarcinoma 16/C (table 3 and fig 2). This is a highly metastatic tumor with a tumor-doubling time of 2–3 days and responds to most of the agents reported to be active against breast cancer in women, eg, doxorubicin, cyclophosphamide, 5-FU, and melphalan (7). Drugs like 6-thioguanine or 6-thioguanine-2′-deoxyriboside were not active against this tumor (7). 3-DG at a dose of 400 mg/kg twice weekly completely inhibited the growth of this tumor, resulting in prolonged remission (22 days) and a significant ILS (150%) in these animals. The results were comparable to or better than those obtained with 5-FU. Corbett et al (7) have reported positive activity for 3-DG in C3H mammary adenocarcinomas 16/C and 13/C at doses of 43 mg/kg/day for 9 days, confirming our results. 3-DG treatment did not produce significant inhibition of the growth of a slow-growing murine colon adenocarcinoma 38 (table 2). In all of the experiments described above, no apparent signs of drug toxicity, such as gross weight loss, diarrhea, or hair loss, were observed at the end of treatments at therapeutic dosages, eliminating any possibility that the tumor growth inhibition may be due to toxic effects on the hosts. 3-DG was ineffective in the treatment of mice bearing Lewis lung carcinoma or B16 melanoma transplants (table 2). It is interesting to note that 3-DG is a potent inhibitor of estrogen receptor-positive mammary adenocarcinoma R3230AC, whereas it stimulated the growth of B16 melanotic melanoma, which also has estrogen receptor in its cytosol and is responsive to antiestrogen therapy (13).

Our studies demonstrate that 3-DG is very active in inhibiting the growth of slow- and rapid-growing solid tumors, especially those that are models for human breast carcinomas. The marginal or total lack of activity of 3-DG in other experimental tumor systems suggests that, unlike common chemotherapeutic agents, 3-DG may not act like a general cytotoxic agent. In view of these data and the data of the National Cancer Institute showing significant inhibition of the MX-1 mammary human tumor xenograft implanted beneath the renal capsule, 3-DG has been approved at the DN2A level by the Decision Network of the National Cancer Institute for development to clinical trial.

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BRIEF REPORTS

Primary Non-Hodgkin's Lymphoma of Bone: Results in 15 Patients Treated by Radiotherapy Combined With Systemic Chemotherapy[1]

Gaetano Bacci,[2] Piero Picci,[2,*] Franco Bertoni,[3] Franco Gherlinzoni,[2] Pierina Calderoni,[2] and Mario Campanacci[2,4]

The authors report on the results obtained in 15 patients with primary non-Hodgkin's lymphoma (NHL) of bone treated by radiotherapy and polychemotherapy; 86% of the patients (13 of 15) were continuously disease-free at a median follow-up of 70 months (42–104). No local recurrence was observed. The necessity of clearly distinguishing primary non-Hodgkin's lymphoma of bone from Ewing's sarcoma is discussed. [Cancer Treat Rep 66:1859–1862, 1982]

Primary non-Hodgkin's lymphoma (NHL) of bone is a rare extranodal lymphoma histologically identical to that arising in lymphoid or soft tissue but presenting initially as a localized solitary bone lesion. This tumor has a significantly different clinical course and a much better prognosis than NHL secondarily affecting bone. Usually, primary NHL of bone is readily distinguished from classic Ewing's sarcoma, even if occasionally a sound histologic diagnosis may be difficult because of features that are intermediate to the two types. The difference in prognosis between these two forms makes the distinction more than academic.

Because the response to radiation is rapid and complete, this modality is preferred by most authors for the local control of primary NHL of bone. In the last decade, on the basis of the results of combined therapy in Ewing's sarcoma, some authors (1,2) have suggested combination chemotherapy even in those cases of NHL of bone where no regional or distant spread of disease is evident.

The purpose of this paper is to report the results obtained in 15 consecutive patients with primary NHL of bone treated at the Bone Tumor Center of the Istituto Ortopedico Rizzoli between 1972 and 1978 by radiotherapy combined with systemic adjuvant polychemotherapy.

MATERIALS AND METHODS

Between 1972 and 1978, 142 patients with histologically confirmed malignant round cell tumors were seen at our center; 96 had Ewing's tumor (16 metastatic and 80 localized), 11 had transitional forms (four metastatic and seven localized), and 35 had NHL of bone (20 metastatic and 15 localized). In the present study we consider only the 15 patients with localized primary NHL of bone.

Patients were entered in the study only if, at onset, there was no evidence of other lesions, as determined by extensive clinical findings, radiologic evidence (chest X-rays and tomograms), isotope bone scan, lymphangiographic studies, and bone marrow biopsy. Staging laparotomy was not done in any patient. The clinical features of these 15 patients are summarized in table 1.

Diagnosis was based on the histologic evaluation of representative sections obtained from open biopsy. In addition to standard staining with hematoxylin and eosin, periodic acid-Schiff stain for glycogen and silver stain for reticulin fibers were used to enhance the accuracy of histologic diagnosis. Patients with debatable histology (ie, transitional to Ewing's tumor) were excluded.

The primary tumor was treated by radiotherapy, started 1 week after biopsy, using ^{137}Cs (patients 1–9) or ^{60}Co (patients 10–15). The total dose of radiotherapy varied from 4000 to 4500 rads according to the site and volume of the tumor, patient age, and the presence of severe soft tissue reaction. Opposing field radiation therapy was given when the tumor was located in a long bone; the doses were 3000–3500 rads to the whole bone and an additional 1000–1500 rads to the tumor site. In

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[1] Received Dec 10, 1981; revised June 3, 1982; accepted June 23, 1982.

[2] Department of Internal Medicine *(G. Bacci)*, Bone Tumor Center *(P. Picci)*, and First Orthopedic Clinic *(F. Gherlinzoni, P. Calderoni, and M. Campanacci)*, Istituto Ortopedico Rizzoli, University of Bologna, Italy.

[3] Department of Pathology, University of Chieti, Italy.

[4] We thank Dr. E. Lorenzi for secretarial help.

**Reprint requests to:* Dr. Piero Picci, Istituto Ortopedico Rizzoli, Centro Tumori Ossei, Via Codivilla 9, 40136 Bologna, Italy.

TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Sex	Bone involved	Histology	Results*	Clinical findings
1	34	M	Femur	Undifferentiated	NED, 104 mos	Bone pain from 2 mos
2	40	M	Femur	Histiocytic	NED, 97 mos	Pathologic fracture
3	41	M	Tibia	Histiocytic	CNS metastasis after 12 mos + 14 mos	Swelling from 1 mo
4	32	F	Ulna	Unclassified	NED, 81 mos	Bone pain from 3 mos
5	50	M	Femur	Histiocytic	CNS metastasis after 10 mos + 12 mos	Bone pain from 6 mos
6	9	M	Tibia	Histiocytic	NED, 74 mos	Bone pain and swelling from 2 mos
7	23	F	L1	Histiocytic	NED, 72 mos	Neurologic symptoms from 1 mo
8	34	M	Femur	Undifferentiated	NED, 70 mos	Pain from 6 mos
9	51	F	Ileum	Histiocytic	NED, 70 mos	Bone pain from 6 mos, swelling from 1 mo
10	18	F	Tibia	Histiocytic	NED, 67 mos	Bone pain and swelling from 3 mos
11	31	M	Ileum	Histiocytic	NED, 65 mos	Pain from 3 mos, anemia
12	21	F	L1	Histiocytic	NED, 65 mos	Neurologic symptoms from 5 mos
13	19	M	Ileum	Histiocytic	NED, 54 mos	Pain from 3 mos
14	52	M	Tibia	Histiocytic	NED, 48 mos	Bone pain from 8 mos
15	36	F	D8	Histiocytic	NED, 42 mos	Bone pain from 3 mos, fever, anemia

*NED = no evidence of disease.

short and flat bones, 3500–4000 rads were delivered to the whole bone, with a boost of 1000 rads to a smaller volume including the tumor itself. In all patients the radiotherapy was delivered over a period of 5–6 weeks (daily dose, 200 rads midplane; 5 fractions/week). Chemotherapy was started on the same day as the biopsy. The initial chemotherapy consisted of vincristine (1 mg/m^2) and cyclophosphamide (500 mg/m^2). Subsequently, adjuvant chemotherapy was given according to the schedule in table 2 and was continued for 2 years. Treatment was delayed if myelosuppression became evident (wbc count < 3000/mm^3 or platelet count < 100,000/mm^3).

All patients had follow-up in our clinic for the detection of recurrent disease, metastasis, and the delayed complications of therapy, every 3 months for 3 years and subsequently every 6 months.

RESULTS

In table 3, the results of this study are reported as well as the results obtained with the same adjuvant

TABLE 2.—Adjuvant chemotherapy scheme (1972–1978)*

	Day 1	Day 2	Day 3	Day 4
Cycle A	ADM (50 mg/m^2)	VCR (1.5 mg/m^2)	CPM (300 mg/m^2)	CPM (300 mg/m^2)
10 consecutive cycles at 21-day intervals				
Cycle B		VCR (1 mg/m^2) +	CPM (800 mg/m^2)†	
32 consecutive cycles at 14-day intervals, starting 21 days after end of cycle A				

*ADM = doxorubicin, VCR = vincristine, and CPM = cyclophosphamide.
†Both drugs were given on same day.

TABLE 3.—Primary localized round cell tumor of bone treated between 1972 and 1978*

	RT + chemotherapy			Surgery ± RT + chemotherapy			Total	
	Treated	NED	Local recurrence	Treated	NED	Local recurrence	NED	Local recurrence
Primary non-Hodgkin's lymphoma (15)	15	13 (86%)	—	—	—	—	13 (86%)	—
Ewing's tumor (80)	48	11 (23%)	17 (35%)	32	19 (56%)	4 (12%)	30 (37%)	21 (26%)
P value		< 0.001			—		< 0.001	

*Excluding transitional forms. RT = radiotherapy, NED = no evidence of disease. Unless otherwise indicated, values = No. of patients.

chemotherapy in 80 patients with localized Ewing's tumor treated by us in the same period (3).

All patients with primary NHL of bone who entered the study completed the chemotherapy program except the two who relapsed. During the treatment, both of these patients presented with cerebral metastases and were treated at other institutions with unknown therapy; they died 12 and 14 months after the diagnosis.

The initial tumor response was rapid and satisfactory in all cases. Radiographic changes indicating improvement occurred by 6–8 weeks in all cases. Of the 15 patients, 13 (86%) remained continuously disease-free for 42–104 months ($\bar{x} = 70$) from the start of therapy. Ten of these patients had follow-up for > 5 years and 12, for > 4 years. The percentage of patients continuously disease-free is by far superior to the percentage observed by us in the 48 patients with Ewing's sarcoma (86% vs 23%) treated in the same period by radiotherapy combined with the identical regimen of adjuvant chemotherapy (table 3).

No local recurrence was observed in our patients with primary NHL of bone. These data contrast with the 35% local recurrence observed in the 48 patients with Ewing's tumor.

Patient tolerance to the chemotherapy was generally good. Only two patients required deviation from the protocol due to pronounced leukopenia or delayed restoration. These two patients were given the entire programmed dose of drugs over a longer period of time. Extrahematologic toxicity was negligible. Despite concurrent use of chemotherapy, toxic effects due to local radiotherapy were moderate; they included edema and induration of the irradiated limb (two patients) and transient infection at the biopsy site (one). No persistent functional impairment or pathologic fracture at the irradiated site was noted over the period considered (3–9 years).

The follow-up time of our patients is still too short to permit evaluation of long-term consequences of the treatment. However, ten patients are alive without any evidence of a secondary tumor at ≥ 5 years from the start of therapy.

DISCUSSION

Today, the treatment of primary NHL of bone varies in different institutions. The accumulated experience with this tumor does not yet allow one to be dogmatic regarding the best treatment. According to some authors (4), when the diagnosis of primary NHL is based on accurate staging including laparotomy the therapy of choice should be radiotherapy only, in view of the potential toxicity of combined chemotherapy. According to other authors (1,5,6), a combination of radiotherapy and chemotherapy is advisable even in those instances in which no regional or distant spread of disease is evident after pathologic staging. Our excellent results with combined therapy and the negligible side effects observed to date have persuaded us to use the latter therapeutic approach.

Although laparotomy has been included in the initial workup at our institute since 1979, we still treat this tumor with combined therapy. However, the chemotherapeutic regimen has been slightly adjusted concerning the duration of treatment (only 12 months) and the number of treatment cycles with doxorubicin (eighty, a total dose of 400 mg/m^2).

Although laparotomy has been included in the initial workup at our institute since 1979, we still treat this tumor with combined therapy. However, the chemotherapeutic regimen has been slightly adjusted concerning the duration of treatment (only 12 months) and the number of treatment cycles with doxorubicin (eight, a total dose of 400 mg/m^2).

1. The overall prognosis of primary NHL of bone is clearly superior to that of Ewing's tumor. This is very important to the performance of a therapeutic trial for Ewing's tumor. For example, if the 15 patients with primary NHL had been included in our trial of adjuvant chemotherapy for localized Ewing's sarcoma, the percentage of patients continuously disease-free, locally treated by radiotherapy alone, would increase from 23% (11 of 48) to 38% (24 of 63).
2. Today, surgical treatment of a primary Ewing's

tumor is being re-evaluated (3,10,11) because (*a*) the use of high-dose radiotherapy (5500–6000 rads) often produces important functional deficit of the limbs, sometimes greater than amputation, and (*b*) there is a high incidence of observed local recurrence after radiotherapy (3,6). On the other hand, according to our results, surgery does not seem to be indicated in the treatment of primary NHL of bone. The radiosensitivity of this tumor permits the delivery of a lower dose of radiotherapy, minimizing local damage. No local recurrence was observed in any of the 15 patients reported, in whom the primary lesion had been treated with only 4000–4500 rads.

3. The site of the localized primary Ewing's tumor is of great importance in determining the prognosis (3,12,13). For example, the prognosis for localized tumor along the skeletal axis is notably worse than for localized tumor in the extremities. On the contrary, in primary NHL the initial site of the tumor does not seem to influence the prognosis. No relapse was observed in our patients with primary localized tumor in the spine or the pelvis.

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BRIEF REPORTS

Fatal Hepatotoxicity Associated With AMSA Therapy[1,2]

Frederick R. Appelbaum* and Howard M. Shulman[3,4]

AMSA is a relatively new chemotherapeutic agent with activity against selected solid tumors and acute leukemia. The usual toxicity from the doses used for solid tumors (120 mg/m^2 every 3 weeks) is mild leukopenia, while at the higher dose levels used for leukemia (50–150 mg/m^2/day for 5 days), there have been reports (1–4) of profound pancytopenia, nausea and vomiting, phlebitis, stomatitis, and hepatic dysfunction. More recently, generalized seizures and cases of ventricular fibrillation temporally related to the AMSA infusion have been observed (5). No case of fatal hepatic toxicity has previously been reported. This case report describes a woman who developed progressive and ultimately fatal liver failure following therapy with AMSA for acute lymphoblastic leukemia.

CASE REPORT

A 44-year-old woman had been diagnosed as having acute lymphoblastic leukemia in March 1975. She achieved an initial remission with prednisone, vincristine, 6-mercaptopurine, and methotrexate and then received central nervous system prophylaxis. Following remission induction, marrow was stored in liquid nitrogen for subsequent autografting. She initially relapsed in July 1979 and was successfully reinduced with L-asparaginase, daunorubicin, vincristine, and prednisone. She subsequently relapsed in January 1980 and was re-treated with prednisone, vincristine, 6-mercaptopurine, and methotrexate without success. In February 1980 she received cyclophosphamide, 1000-rad total-body irradiation, and her autologous remission marrow that was stored 4$^1/_2$ years previously, resulting in a complete remission lasting 10 months. She was then treated with methotrexate and L-asparaginase, but in April 1981 she was again in relapse. The patient was then admitted to the University of Washington Medical Center for experimental therapy with AMSA.

On admission she appeared well with normal vital signs and had no abnormalities during physical examination. Her weight was 57.7 kg. A cbc showed a hematocrit of 34%, a wbc count of 1500/mm^3 (comprised mostly of lymphoblasts), and a platelet count of 18,000/mm^3. Biochemical studies revealed normal serum electrolytes; BUN, 9 mg/100 ml; creatinine, 0.7 mg/100 ml; bilirubin, 0.7 mg/100 ml; SGOT, 25 units/L (normal, 0–25); alkaline phosphatase, 67 units/L (normal, 20–105); amylase, 0.8 units/L (normal, 0–3); and prothrombin time, 100% activity. Chest X-ray and electrocardiogram were normal.

The patient was given AMSA at a dose of 120 mg/m^2 diluted in 500 ml of 5% dextrose in water and infused over 2 hours for 5 consecutive days. She did well for the first 12 days after the initiation of chemotherapy, with only the expected myelosuppression. On Day 13 she became febrile (38.8°C) and started receiving tobramycin and ticarcillin. By Day 16 the fever had abated, and she remained afebrile until 2 days before her death on Day 27. On Day 17 her liver function tests first became abnormal, with a bilirubin of 2.3 mg/100 ml and an alkaline phosphatase of 286 units/L. Over the next 10 days (from Day 17 to Day 27), liver dysfunction progressed to liver failure, which was fatal. The patient's bilirubin steadily increased from 2.3 to 16.5 mg/100 ml; alkaline phosphatase increased to 460 units/L and then plateaued; prothrombin time fell to 15% activity, and SGOT remained normal. A test for hepatitis B antigen was negative. She developed progressive ascites and her weight rose from 58.7 to 69.5 kg despite the use of albumin, furosemide, and spironolactone. On Day 23, the patient developed signs of hepatic encephalopathy,

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[1] Received Jan 29, 1982; revised June 14, 1982; accepted June 24, 1982.

[2] Supported by Public Health Service grant CA-18029 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. F. R. Appelbaum is supported in part by a Junior Faculty Clinical Fellowship from the American Cancer Society.

[3] Division of Oncology, Department of Medicine, University of Washington School of Medicine, and Fred Hutchinson Cancer Research Center, Seattle.

[4] We thank Dr. Karmal Ishak of the hepatic pathology branch of the Armed Forces Institute of Pathology, Washington, DC, for reviewing the liver histopathology.

*_Reprint requests to:_ Frederick R. Appelbaum, MD, Division of Oncology, Department of Medicine, Fred Hutchinson Cancer Research Center, 1124 Columbia St, Seattle, WA 98104.

with diminished mental status and asterixis. She was treated with oral neomycin and lactulose enemas. Over the next 2 days, the encephalopathy progressed to coma, and for the first time her creatinine began to rise. From Day 25 to Day 27 she remained comatose with increasing renal failure, and she died on Day 27.

An autopsy was performed 8 hours after her death. Findings included a hypoplastic marrow with no evidence of leukemia and evidence of fungal infection with *Candida albicans* with several colonies in the lungs, spleen, pleura, kidney, and liver. The liver weighed 2950 g, was of normal color, and was spotted with several white fibrous nodules. Histologically, the liver lobules were composed of markedly disrupted hepatic cords, with loss of cohesion and radial orientation (fig 1). The enlarged and bizarre hepatocytes lying in the dilated sinusoids had rounded to angulated outlines. Many hepatocytes, especially in the centrilobular zones, had cytoplasmic ballooning with increased amounts of ceroid and hemosiderin pigment. Hepatocyte nuclei were enlarged, were often binucleate, and contained prominent nucleoli. Portal triads were mildly expanded with a lymphocytic infiltrate and bile ductule proliferation. Rare bile thrombi were present in the ductules. The terminal hepatic venules and sublobular veins showed no change of veno-occlusive disease on connective tissue stains. Even allowing for some changes from autolysis, the overall impression was of a diffuse and severe hepatocellular injury.

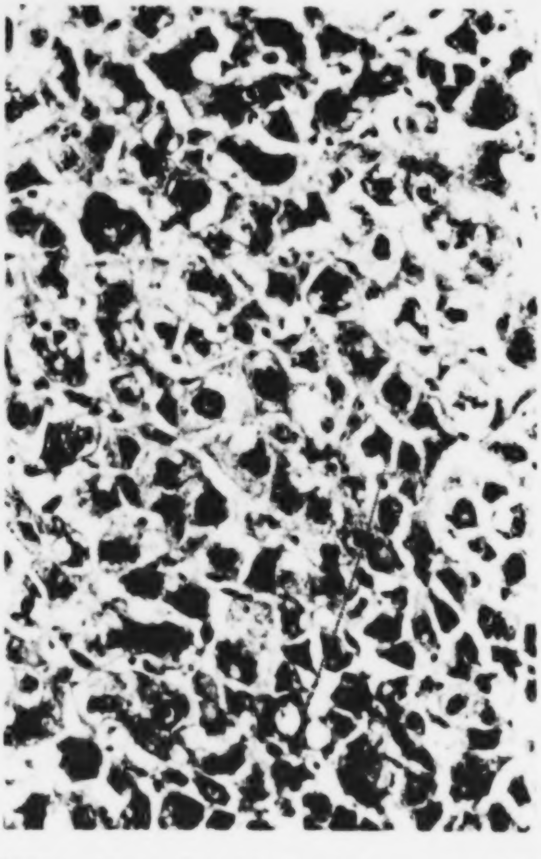

FIGURE 1.—Autopsy section of liver reveals disarray of liver cords with sinusoidal dilation and prominent Kupffer's cells. Hepatocytes have marked anisonucleocytosis, nuclear hyperchromasia with prominent nucleoli, cytoplasmic ballooning with marked ceroid and hemosiderin accumulation, and mild centrilobular cholestasis (Mason trichrome, × 250).

DISCUSSION

Hepatic toxicity associated with AMSA was predicted by the preclinical toxicology studies but was not noted in the phase I trials, in which hematopoietic toxicity was the dose-limiting factor (1,2). However, studies using much higher doses of AMSA for the treatment of acute leukemia demonstrated that AMSA is probably hepatotoxic. Arlin et al (3) reported hepatotoxicity in eight of 31 patients treated with AMSA at a dose of 200 mg/m^2/day for 5 days. As in our case, the evidence for hepatotoxicity was mostly elevations in bilirubin and alkaline phosphatase and was delayed for 2–3 weeks after AMSA was administered. Legha et al (4) reported elevations in bilirubin in 14 of 62 patients treated with AMSA but felt the hyperbilirubinemia may have been due in part to severe infection.

Although it is difficult to define the cause of liver dysfunction in patients with acute leukemia treated with multiple drugs, it seems most likely to us that AMSA was a major cause of the hepatotoxicity that led to this patient's death. The only other medications she received during this hospital course were methadone, ticarcillin, tobramycin, furosemide, spironolactone, docusate, and Tylenol. She had received all of these during other admissions without problems. No structural abnormalities were seen during autopsy. While she had disseminated candidiasis, the extent of the lesions was not sufficient to account for the degree of hepatic dysfunction, nor does it explain the severe hepatocellular injury seen at autopsy. She had received extensive antileukemic treatment prior to receiving AMSA, and this may have made her more susceptible to hepatic damage.

AMSA is an important new antileukemic agent. However, the occurrence of fatal hepatotoxicity in our patient should alert physicians that liver function abnormalities arising during the course of AMSA chemotherapy may be due to this drug and also that AMSA should be used with caution in patients with known hepatic dysfunction.

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BRIEF REPORTS

Response to Oophorectomy After Tamoxifen Failure in a Premenopausal Patient[1,2]

Alfred M. Kalman, Thomas Thompson, and Charles L. Vogel[3,*]

Previous reports of tamoxifen therapy in premenopausal patients have failed to show responses to ovarian ablation if the patients did not respond to tamoxifen. A case is presented of a response to oophorectomy in a tamoxifen nonresponder and the literature is reviewed. [Cancer Treat Rep 66:1867–1868, 1982]

During the proceedings of a symposium on antiestrogen therapy for hormone-dependent tumors, two groups of investigators (1,2) reported on the use of tamoxifen as primary hormonal therapy in premenopausal patients with metastatic breast cancer. In these two studies, the response to tamoxifen therapy was a strong predictor of a patient's future response to ovarian ablation, with none of 17 tamoxifen failures responding to subsequent ovarian ablation. The present report details a case of a premenopausal woman who failed to respond to tamoxifen therapy, but subsequently had a response to bilateral salpingoophorectomy (BSO).

CASE REPORT

The patient, a 31-year-old menstruating Cuban woman, underwent a left modified radical mastectomy in January 1980 for a $T_3N_{1a}M_0$ poorly differentiated infiltrating ductal carcinoma; 12 of 26 axillary nodes were positive. Estrogen-receptor (ER) assay performed at the time of mastectomy was 102.2 femtomols/mg of cytosol protein and a metastatic work-up was negative. In view of the patient's nodal status, she was entered in a Southeastern Cancer Study Group protocol and randomized to receive 5000 rads of postoperative adjuvant radiotherapy to be followed by six courses of adjuvant cyclophosphamide, methotrexate, and 5-FU (CMF) chemotherapy. The patient was given four courses of CMF and was then lost to follow-up until December 1980 when she had a recurrence with epidural cord compression from bony metastases in the L_1–L_3 region. Restaging workup performed at this time also revealed lytic lesions in the skull, bilateral ilia, upper femora, pubic rami, and right sacrum. A chest X-ray showed a left pleural effusion extending one-third up the left hemithorax. A liver scan was normal and serum alkaline phosphatase was 115 IU/L. The patient was treated with lumbar spine irradiation and started receiving tamoxifen at a dose of 10 mg orally twice a day, with repeat chest X-ray 3 weeks later revealing an increase in the left pleural effusion and no significant change in bone X-rays (pelvis, femora, and skull). The dose of tamoxifen was then increased to 20 mg orally twice a day, and repeat films 8 weeks later showed continued progression of the left pleural effusion so that it now occupied almost the entire left hemithorax. The patient's weight had decreased from 150 to 130 lbs since the start of tamoxifen therapy. Bone X-rays of the skull, pelvis, and femora were again without change. Tamoxifen was discontinued, and the patient was admitted for thoracocentesis and tetracycline sclerosis of her left pleural space. The pleural fluid was noted to be an exudate with negative cytology. Two weeks after tamoxifen was discontinued, a new right pleural effusion was noted on chest X-ray, and the patient underwent sclerosis of her right pleural space followed by a BSO. The fluid from the right pleural space was not sent for diagnostic workup. Four weeks after BSO, a repeat chest X-ray showed the right and left pleural effusions to be unchanged from X-rays taken after tetracycline sclerosis; however, sclerotic repair of the lytic lesions of the ilia, femora, skull, right sacrum, and bilateral pubic rami were noted. Follow-up films during the next 4 months of care revealed continued improvement in all

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[1] Received Feb 8, 1982; revised June 11, 1982; accepted June 16, 1982.

[2] Supported in part by grant 5207 from the American Cancer Society.

[3] Departments of Oncology (*A. M. Kalman* and *C. L. Vogel*) and Radiology (*T. Thompson*), University of Miami School of Medicine, FL.

[*] *Reprint requests to:* Dr. Charles L. Vogel, Division of Breast Cancer, Department of Oncology D8-4, University of Miami School of Medicine, PO Box 016960, Miami, FL 33101.

lytic lesions mentioned and stabilization of both pleural effusions. Serial determinations of alkaline phosphatase ranged from 109 to 143 IU/L. With objective improvement in the lytic lesions, the patient noticed concomitant decrease in skeletal pain and markedly improved Karnofsky performance status (from 60% before BSO, to 80%). Narcotic analgesic (Empirin #4) requirement was reduced from eight to three tablets daily and the patient's weight stabilized at 130 lbs with no anorexia.

DISCUSSION

A variety of potential hormonal manipulations are available to treat advanced breast cancer. The classical approach to initiating hormonal therapy in ER-positive premenopausal patients with disseminated breast cancer has been surgical ablation of the ovaries. Current preliminary evidence suggests that the antiestrogen tamoxifen may have remisssion-inducing potential and remission duration similar to those of surgical ovarian ablation (3-9). It was therefore elected to initiate hormonal therapy with tamoxifen in our patient. Although we were not able to document a positive cytology from the left pleural effusion, clinically, the patient did not exhibit any signs or symptons to suggest a diagnosis other than malignancy. The marked progression of the left pleural effusion with tamoxifen and the appearance of a new right pleural effusion with tamoxifen withdrawal (10) necessitated institution of a different form of therapy. Even though our patient did not respond to the first hormonal manipulation, a BSO was done and definite objective and symptomatic improvement was noted in the ensuing 4 months.

Previous reports of tamoxifen therapy in premenopausal patients have failed to show responses to ovarian ablation if the patient did not respond to tamoxifen. Our literature review indicates that there have been only 17 patients who have had ovarian ablation after failing tamoxifen; none of these patients responded. However, of the 17 nonresponders, only four were documented to have ER-positive tumors. The remaining patients (eight, ER unknown; four, ER negative; and one, ER borderline) may have had intrinsically hormonally resistant tumors and thus may not have been an ideal population to test the effectiveness of sequential hormonal maneuvers.

Although patients who do not respond to one hormone have a low probability of benefiting from any other endocrine treatment, it has been shown in tamoxifen nonresponders that the subsequent use of androgens or hypophysectomy may yield a response (11). It therefore seems reasonable to assume that a similar change in the hormonal milieu (ie, ovarian ablation) might also be efficacious.

It is of interest to note that while receiving tamoxifen, our patient continued to have regular menstrual cycles, indicating an incomplete antiestrogen effect. Although it is clear that tumor shrinkage by tamoxifen can occur without achieving any irregularity in the menses, one might speculate that patients who manifest evidence of estrogen deprivation while receiving antiestrogen therapy may have a higher rate of response, as suggested by Pritchard et al (2). It is possible that our patient's failure to respond to tamoxifen was secondary to an inability to exert sufficient antiestrogenic effect at the level of the tumor as well as the uterus at the doses prescribed.

Since initial submission of this paper, a study was reported by Hoogstraten (12) also challenging the initial observations of Pritchard et al (2). In Hoogstraten's study, response to tamoxifen therapy did not predict for future response to ovarian ablation because five of 22 premenopausal tamoxifen nonresponders subsequently had a response to BSO, whereas none of 12 tamoxifen responders who underwent BSO at relapse had a response. This study is not strictly comparable to prior studies, however, since tamoxifen was continued after ovarian ablation. Although no generalizations can be derived from our single case report, we still consider oophorectomy as a therapeutic option in premenopausal tamoxifen nonresponders.

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BRIEF REPORTS

Phase Evaluation of Bisantrene in Patients With Renal Cell Carcinoma[1,2]

J. William Myers,[3] Daniel D. Von Hoff,[3,*] Charles A. Coltman, Jr,[3] John G. Kuhn,[3] David Van Echo,[4] Saul Rivkin,[5] and Robert Pocelinko[6,7]

Bisantrene is a new anthracene bishydrazone derivative thought to function as a DNA intercalating agent. In recently reported phase I studies, responses to this drug were seen in patients with a variety of tumors including renal cell carcinoma (1,2). In addition, in vitro antitumor activity was noted for bisantrene against renal cell carcinoma in the human tumor cloning system. With that assay, in vitro responses ($\geq 70\%$ decrease in tumor colony-forming units) were noted in four of 18 patients' tumors growing in culture (3). This background, in addition to the known resistance of this tumor to established chemotherapeutic agents, served as an impetus to conduct a phase II study of bisantrene in patients with renal carcinoma. Our objectives included: (*a*) establishing the efficacy of bisantrene as a single agent used in a single-dose intermittent schedule and (*b*) evaluating the utility of the human tumor cloning system for screening for phase II clinical activity of a new compound.

PATIENTS AND METHODS

Forty-two patients with advanced renal cell carcinoma were entered in this study between August 1980 and September 1981. Histologic confirmation was obtained in 41 patients. An additional patient with typical angiographic findings of a renal hypervascular mass and inferior vena cava tumor thrombus was included. Patients with clearly measurable disease, adequate bone marrow, and adequate hepatic (bilirubin < 2.0 mg/dl) and renal (creatinine < 2.0 mg/dl) functions were selected for the study, regardless of performance status (PS). The presence of an unstable cardiac rhythm or recent congestive heart failure was grounds for exclusion. Bisantrene was administered as an iv infusion in 300–500 ml of 5% dextrose in water over 1–2 hours every 3 weeks. The drug was given either by a peripheral vein or via a Broviac central catheter. Initially, patients received 260 mg/m^2, with subsequent dose increase (10%) or decrease (20%–50%) depending on the absence or presence and severity of toxic effects. Cbcs were determined weekly, and hepatic and renal function tests were obtained prior to each infusion. Later entries (six patients) with adequate marrow reserve received an initial dose of ≥ 280 mg/m^2. In addition, at the invesigators' discretion, patients were pretreated with parenteral Benadryl and/or corticosteroids in attempts to obviate allergic-like reactions during iv infusions of bisantrene.

An adequate trial was defined as two doses of drug with subsequent follow-up. Standard response criteria were applied. A complete response was defined as the disappearance of all clinical evidence of active tumor for at least 4 weeks. A partial response was defined as a $\geq 50\%$ decrease in the sum of the products of two perpendicular diameters of the measured lesions. Patients with liver metastases had to have a 30% reduction in the sums of the liver span measurements below each costal margin. These reductions in tumor or organ size had to last at least 4 weeks, with no simultaneous increase in size or simultaneous appearance of new lesions. Stable disease was defined as a steady state or response less than partial remission maintained for a minimum of 8 weeks. Disease progression was defined as a 25% increase in the size of any measurable lesion or the appearance of new lesions.

The study consisted of 30 males and 12 females, with a median Southwest Oncology Group PS of 1 (range, 0–4). The median age was 64 years (range, 28–84). Nine-

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[1] Received Mar 1, 1982; revised June 28, 1982; accepted July 12, 1982.

[2] Supported by a grant from the Medical Research Division, American Cyanamid Co.

[3] Department of Medicine, Division of Oncology, University of Texas Health Science Center at San Antonio.

[4] University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.

[5] Tumor Institute of Swedish Hospital Medical Center, Seattle, WA.

[6] Medical Research Division, American Cyanamid Co, Lederle Laboratories, Pearl River, NY.

[7] We thank the following physicians for contributing to this study: Charles Mims, John Sandbach, Brent Ellis, Victorio Rodriguez, Stephen Cohen, Donald Oines, David Gordon, and Paul Ritch.

**Reprint requests to:* Daniel D. Von Hoff, MD, Department of Medicine, Division of Oncology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78284.

teen patients had had prior hormonal therapy, four had had prior chemotherapy, and five had had both. Fourteen patients had had no prior systemic therapy. Sites of indicator lesions included lung (24 patients), bone (nine), nodes (eight), soft tissue (five), and liver (four).

RESULTS

Antitumor Activity

Five patients were excluded from response analysis. Two patients died of progressive disease on Days 6 and 12 of their first bisantrene cycle, two were lost to follow-up after the second drug infusion, and one refused further therapy after the first infusion. Two of the 37 evaluable patients had responses. A 51-year-old male (PS = 0) with liver metastases and prior hormonal therapy experienced a partial response lasting 10 weeks, and a 51-year-old female (PS = 0) with lung metastases and prior drug and hormonal therapy was removed from study in partial remission (10+ weeks) after an anaphylactoid reaction. Another patient, a 67-year-old female (PS = 0) with prior hormonal therapy, had near resolution of lung nodules while bone disease progressed after 12 weeks of therapy. Twelve patients had disease stabilization for a minimum of 8 weeks (range, 8–39). Twenty-two patients had clear disease progression during bisantrene therapy.

A total of 169 bisantrene infusions were given. The median dose administered was 280 mg/m^2 (range, 160–370). The median number of infusions per patient was four (range, two to 14). Of the 37 evaluable patients, 28 experienced dose-limiting toxic effects prior to going off study.

Toxicity

Toxicity, assessed in 35 patients, is summarized in table 1. While granulocytopenia (nadir counts determined on Day 8) appeared to be the major dose-limiting effect, there was no evidence that this was cumulative (cumulative dose median, 1040 mg/m^2; range, 520–3860). Further, no clinical cardiac toxicity was evident in this group of patients.

Signs and symptoms comprising an anaphylactoid reaction were seen in six patients; they were abrupt and clinically serious and included hypotension, diaphoresis, shortness of breath, chills, nausea, and vomiting. Other inconstant symptoms in these six episodes were back pain, fever, and a flushing sensation. All six patients had received multiple bisantrene infusions (range, three to 13), and there were no uniform prodromal symptoms during their prior infusions. Further, premedicating five of six of these patients with Benadryl and/or corticosteroids did not prevent the subsequent intrainfusion reaction.

TABLE 1.—Toxicity of bisantrene (35 patients)

Toxic effect	No. of patients (%)
Granulocytopenia (polymorphonuclear neutrophils/mm^3)	
1000–1500	9 (26)
500–999	13 (37)
250–499	5 (14)
0–249	1 (3)
Anemia (Hgb, g/100 ml)	
9.0–9.9	12 (34)
7.0–8.9	8 (23)
Thrombocytopenia (platelets/mm^3)	
75,000–99,999	1 (3)
Nausea/vomiting	12 (34)
Local reactions*	9 (26)
Fever	9 (26)
Chills	5 (14)
Anaphylactoid reaction	6 (17)
Miscellaneous	
Mucositis	1 (3)
Urticaria	1 (3)
Flushing	1 (3)
Diarrhea	1 (3)
Chest pain	1 (3)
Hiccoughs	1 (3)

*Extravasation with subsequent ulceration in 2 patients.

Local reactions were noted in 26% of the patients receiving the drug by peripheral vein. These reactions included pain at injection site without objective findings, discoloration of vein proximally, and local angioedema. Two patients had extravasation with subsequent ulcer formation which healed in 6 weeks. There were no drug-related deaths.

DISCUSSION

Two of 37 evaluable patients (5%) experienced partial remissions during this study, indicating minimal activity of bisantrene against renal cell carcinoma when administered on this schedule. This trial appears adequate in light of 28 of 37 patients experiencing dose-limiting toxic effects and lack of enhanced activity when bisantrene was administered at higher starting doses ($\geq$ 280 mg/m^2) in six patients at the conclusion of the study.

The occurrence of the anaphylactoid reactions may have contributed to this lack of activity. Of the six patients removed from the study subsequent to these anaphylactoid reactions, one had a partial response approaching complete remission, one had stable disease approaching a partial response, and the remainder had stable disease. The etiology of this reaction remains to be defined.

The local reactions noted with bisantrene administration may be related to limited solubility and possible luminal precipitation of the drug (4). Use of antihistamines and steroids to reduce the incidence of this local phenomenon is being studied in ongoing trials. Central infusions may obviate these problems.

Bisantrene has demonstrated no significant antitumor activity clinically with the schedule and dose utilized. This is disappointing in light of the screening data in the cloning assay where four of 18 patients' tumors showed an in vitro response to the drug. Reasons for this difference between in vitro and in vivo results need to be defined to further assess the usefulness of the assay for in vitro phase II trials.

Despite the low response rate noted in the present study, it is possible that phase II studies using bisantrene in other dosing schedules may reveal antitumor activity against renal cell carcinoma. Such trials are ongoing in other institutions.

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Mitomycin, Hexamethylmelamine, and Vindesine as Second-Line Treatment for Small Cell Cancer of the Lung[1]

Rudolf A. Joss,* Kurt W. Brunner, and Aron Goldhirsch[2]

Combination chemotherapy has considerably changed the outlook for patients with small cell lung cancer, and in several reported series there is now a small fraction of long-term survivors. However, 80%–90% of the patients ultimately die of their disease. The results of second-line chemotherapy after relapse are disappointing. Mitomycin, hexamethylmelamine, and vindesine have been reported to be active in the treatment of small cell lung cancer (1–4).

In the present study, 13 male patients with relapsing small cell lung cancer (table 1) were treated with mitomycin (10 mg/m^2 iv on Day 1), hexamethylmelamine (100 mg/day orally on Days 1–14 and 21–36), and vindesine (3 mg/m^2 iv on Days 1, 8, 21, and 28); this cycle was repeated on Day 43.

One patient experienced a partial remission, with dramatic shrinkage of liver metastases lasting for 66 days. Six patients had disease stabilization for a median

TABLE 1.—Patient characteristics, response, survival, and toxicity

	No. of patients			
	Response			
Characteristic	Partial	No change	Disease progression	Total
Median age in yrs (range): 62 (40–70)				
Performance status				
0	0	1	2	3
1	0	1	2	3
2	1	4	2	7
Stage				
Limited disease	0	3	4	7
Extensive disease	1	3	2	6
Previous chemotherapy				
3 drugs	1	2	0	3
4–6 drugs	0	2	4	6
> 6 drugs	0	2	2	4
Overall response	1	6	6	—
Median survival in mos (range): 3.5 (1.5–6.0)				
Hematologic toxicity				
Leukocyte count nadir (cells/mm^3)				
Median (range): 1900 (800–5000)				
No. of patients with < 2000: 7				
Platelet count nadir (cells/mm^3)				
Median (range): 105,000 (40,000–230,000)				
No. of patients with < 75,000: 5				

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[1] Received Feb 16, 1982; revised June 17, 1982; accepted June 29, 1982.

[2] Institute for Medical Oncology (*R. A. Joss* and *K. W. Brunner*), Ludwig Institute for Cancer Research (*A. Goldhirsch*), University of Berne, Inselspital, Switzerland.

**Reprint requests to:* Rudolf A. Joss, MD, Institut für Medizinische Onkologie der Universität, Inselspital, 3010 Berne, Switzerland.

of 77 days, and six patients showed disease progression. Of the seven patients with a response or stable disease, two showed further disease progression locoregionally whereas five progressed with liver (three patients) or bone (two patients) metastases. Of the six nonresponders, four progressed with distant metastases (liver, two patients; bone, one; and brain, one), and two progressed locoregionally. Median survival for all patients was 3.5 months (1.5–6.0). The seven patients with a response or stable disease survived for a median of 3.5 months (2.5–4+) and the nonresponders for a median of 3.0 months (1.5–6.0). Hematologic toxicity in these heavily pretreated patients was manageable, with a median leukocyte count nadir of 1900/mm^3 and a median platelet count nadir of 105,000/mm^3 (table 1). One patient showed a transient rise of serum creatinine to 1.69 mg/100 ml. Six patients experienced slight nausea and vomiting. Moderate signs of neurotoxicity with paresthesias and muscle weakness were observed in eight patients.

We conclude that the combination of mitomycin, hexamethylmelamine, and vindesine has acceptable toxicity but no major impact on the natural course of relapsing small cell lung cancer. The activity of mitomycin in small cell lung cancer needs to be further defined.

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CLINICAL TRIALS SUMMARIES

Aziridinylbenzoquinone (AZQ) in Advanced Breast Cancer: A Cancer and Leukemia Group B Phase II Trial[1]

Daniel R. Budman,* Arlene Forastiere, Marjorie Perloff, Michael Perry, Joseph Aisner, Vivian Weinberg, and William Wood[2]

AZQ is a lipophilic aziridinylbenzoquinone that has entered phase II trials (1). Because of evidence of cytolytic activity in human mammary xenografts (2) and murine tumor systems (3), the Cancer and Leukemia Group B (CALGB) initiated a phase II trial in patients with advanced mammary carcinoma.

Female patients with histologically documented breast adenocarcinoma, who were not candidates for surgical resection, radiation therapy, or standard chemotherapy, were studied. No patients had been exposed to more than two chemotherapeutic regimens.

CALGB criteria for entry in the study included measurable disease, ambulatory status (CALGB performance status ≤ 3), total wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, serum bilirubin ≤ 1.5 mg/dl, serum creatinine ≤ 1.5 mg/dl, no prior therapy for 4 weeks before entry, anticipated survival > 2 months, and informed consent.

AZQ was dissolved in 100 ml of normal saline and given to study patients at a dose of 30 mg/m^2 by iv infusion over 30 minutes, once every 3 weeks (1). If there was evidence of minor hematologic toxicity (wbc count of 3500–3999/µl) on the day of re-treatment, the drug dose was reduced by 25%. For more significant toxicity (wbc count of 2500–3499/µl and/or platelet count of 75,000–99,999/µl), the dose was reduced by 50%. For greater toxicity, the drug was withheld, and peripheral blood cell counts were performed at weekly intervals until acceptable for treatment.

Patients had a weekly cbc and platelet count. Repeat serum liver function tests, creatinine, BUN, and glucose were done every 21 days.

Of 41 patients entered, 35 were eligible for study; four did not meet eligibility criteria, and two violated the study design. Eighty-nine percent of the patients had previously failed anthracycline therapy (table 1).

Toxicity was mainly hematologic, causing leukopenia or thrombocytopenia (table 1). Patients became progressively more anemic with repetitive therapy, showing a statistically significant drop in mean hemoglobin

TABLE 1.—Pretreatment characteristics and study results

Characteristics and results	% of patients
Eligible for study: 35 patients	100
Initial performance status	
0	26
1	23
2	48
3	3
Age in yrs	
Mean: 58	
Range: 34–58	
Prior chemotherapy	
1 combination	26
2 combinations	74
No prior anthracycline	11
Dominant site of disease	
Visceral	63
Osseous	17
Soft tissue	20
Cycles of therapy	
Mean No.: 1.6	
> 3	23
Toxic effects	
Anemia (Hgb, 7–9.0 g/dl)	33
Leukopenia (wbcs/µl)	
2000–4000	40
1000–1999	23
< 1000	17
Thrombocytopenia (platelets/µl)	
50,000–100,000	23
20,000–49,999	27
< 20,000	17
Nausea/vomiting	11
Complete/partial responses: 0	
Stabilization/minor improvement: 7	
Disease progression: 28	

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[1] Received Apr 1, 1982; revised June 28, 1982; accepted July 14, 1982.

[2] Cancer and Leukemia Group B.

**Reprint requests to:* Daniel R. Budman, MD, Division of Medical Oncology, North Shore University Hospital, 300 Community Dr, Manhasset, NY 11030.

(11.8 to 10.7 g/dl) by Day 21 of the study.

Twenty-three patients had obvious disease progression. One patient refused evaluation at Day 21, and four died within 2 weeks of the start of the protocol (scored as nonresponders).

The finding of no major antitumor response to this drug in 35 patients precludes a 15% response frequency (with 99% confidence) in pretreated patients with breast cancer.

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CLINICAL TRIALS SUMMARIES

Combination Chemotherapy With Vincristine, Carmustine (BCNU), and Bleomycin in Patients With Squamous Cell Bronchial Carcinoma Resistant to Cyclophosphamide, Doxorubicin, Methotrexate, and Procarbazine (CAMP)[1]

A. Veronesi,* M. D. Magri, M. G. Trovò, U. Tirelli, E. Galligioni, S. Tumolo, and E. Grigoletto[2]

The activity of the CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) regimen in metastatic non-oat cell bronchial carcinoma (NOBC) was identified by Bitran et al (1) and confirmed by other authors (2,3). However, even for responders, median survival does not exceed 12 months, indicating the need for effective non-cross resistant second-line regimens. Unfortunately, previous treatment reduces the responsiveness to antitumor drugs in NOBC (4). A combination of vincristine (VCR), carmustine (BCNU), and bleomycin (BLM) was prospectively evaluated at our division from July 1979 to December 1980 as second-line chemotherapeutic treatment for patients with advanced NOBC who failed CAMP chemotherapy.

Thirty-two patients with measurable lesions were treated; all of them had squamous cell carcinoma in progression after cobalt 60 radiotherapy to the tumor and regional lymph nodes (45 grays in 15 sessions) and CAMP chemotherapy at the standard dosage described by Bitran et al (1) (median, four cycles). Chemotherapy consisted of VCR (1.2 mg/m^2 iv) and BCNU (100 mg/m^2 iv) given at 0 hours and BLM (5 mg im) given at 6, 30, 54, 78, and 102 hours. VCR and BLM were repeated every 2 weeks; BCNU was repeated every 6 weeks. Chemotherapy was continued until disease progression. Response was evaluated according to standard criteria and was expressed as complete response, partial response, minimal response, unchanged, and disease progression. In 15 patients the indicator lesion was in an irradiated area. Patient characteristics and response to treatment are reported in table 1. Chemotherapy was well-tolerated and treatment was never discontinued because of toxicity.

Our results are similar to those reported by Mintz et al (5), who could not demonstrate any clinical activity of a combination of hexamethylmelamine, BLM, and

TABLE 1.—Characteristics of patients studied

Characteristics	No. of patients
Patients entered in study	32
Patients evaluable	27
Median age: 60 yrs (range, 43–72)	
Sex	
Male	25
Female	2
Median Karnofsky performance status: 75 (range, 40–90)	
Sites of disease	
Locoregional relapse	16
Lymph node metastases	8
Bone metastases	2
Skin metastases	1
Median No. of cycles: 4.5 (range, 1–11)	
Median % of theoretic dose of drugs administered (range)	
VCR: 100 (60–100)	
BCNU: 100 (40–100)	
BLM: 100 (70–130)	
Responses	
Complete	0
Partial	0
Minimal	2
Unchanged	18
Disease progression	7
Sites of disease progression	
Local	6
Lymph nodes	2
Bones	1
Toxicity	
Leukopenia (< 4000 cells/mm^3)	3
Fever	5
Skin pigmentation	1
Pulmonary toxicity at spirometry	3
Mucositis	3
Paresthesias	9
Median survival: 5 mos (range, 1–16)	

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[1] Received Apr 15, 1982; revised June 24, 1982; accepted July 12, 1982.

[2] Division of Radiotherapy and Medical Oncology, General Hospital, Pordenone, Italy.

**Reprint requests to:* Dr. A. Veronesi, Division of Radiotherapy and Medical Oncology, General Hospital, 33170 Pordenone, Italy.

lomustine (CCNU) in CAMP-resistant patients. The use of conventional agents in chemotherapy-refractory bronchial squamous cell carcinoma does not seem to be of clinical utility, and the use of investigational agents appears to be indicated in this situation.

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LETTERS

Leukopenia Associated With Aminoglutethimide Therapy: A Case Report

Several articles within the past year (1,2) have reported on the efficacy and safety of aminoglutethimide compared with surgical adrenalectomy for the treatment of metastatic breast cancer in postmenopausal women. The major side effects of aminoglutethimide include lethargy, a rash, ataxia, and orthostatic dizziness, which rarely necessitate discontinuation of the drug. A search of the literature located only one case report implicating aminoglutethimide as a cause of pancytopenia (3). I report here a second case of severe neutropenia related to the use of this drug.

The patient, a 69-year-old woman, presented to another hospital in December 1980 with a pathologic fracture of the right femur. Biopsy of this site as well as of a fungating tumor of the right breast revealed infiltrating ductal carcinoma. Estrogen and progesterone receptors were strongly positive. X-rays revealed the tumor to be widely metastatic to bone, especially the ribs. The patient then underwent a simple mastectomy of the right breast. The femur was pinned and irradiated to a total dose of 3900 rads. Past medical history revealed a hysterectomy and bilateral oophorectomy in 1939.

Tamoxifen at a dose of 10 mg twice a day orally was started, with symptomatic relief initially. However, by September 1981 the disease had progressed and it was elected to begin aminoglutethimide at a dose of 250 mg twice a day orally and cortisone at a dose of 100 mg orally (60 mg at bedtime and 20 mg twice a day). Two weeks later, the dose of aminoglutethimide was increased to 250 mg four times a day, and the dose of cortisone was reduced to 40 mg orally (20 mg at bedtime and 10 mg twice a day). Cbc at that time revealed a wbc count of 4100/mm^3, with 63 segmented band cells, 30 lymphocytes, four monocytes, one eosinophil, and two basophils. Hemoglobin was 11.3 g/dl and hematocrit was 34%. One month later, the patient returned for routine follow-up, and the cbc revealed a wbc count of 2.3/mm^3, with 60 segmented band cells, 33 lymphocytes, two basophils, three eosinophils, and two band cells. At her second monthly visit 10 weeks after initiation of therapy, the wbc count was 1000/mm^3, with eight segmented band cells, 84 lymphocytes, six monocytes, and two eosinophils. Hemoglobin was 11.8 g/dl and hematocrit was 35%; platelet count was adequate. The patient felt well and had no complaints except for left infrascapular pain, which she had had throughout her disease course but for which she took no medication. A bone marrow biopsy from the left iliac crest revealed new bone growth, myelofibrosis, and metastatic breast carcinoma. Aminoglutethimide was discontinued and cortisone was maintained. When the patient returned the following day with a urinary tract infection and probable gram-negative sepsis, she started receiving cefazolin and gentamicin, but this treatment was changed to ampicillin in 2 days when the culture results were obtained. Her course was complicated by oral thrush, which responded to topical nystatin. The wbc count nadir was 400/mm^3 on the second hospital day, with no granulocytes seen. Seven days after discontinuation of the aminoglutethimide, the wbc count had begun to increase and by Day 12 it had returned to pretreatment levels (3400/mm^3, with two band cells, 25 segmented band cells, 52 lymphocytes, two myelocytes, and three metamyelocytes), where it has remained pending further therapy.

The patient described by Lawrence et al (3) was receiving triamterene for blood pressure control in addition to aminoglutethimide. The bone marrow recovered after discontinuation of the aminoglutethimide, despite continuation of the triamterene. The patient in the present report was receiving no medication except aminoglutethimide and cortisone (50 mg daily). Underlying malignancy and metastatic involvement of the marrow was undoubtedly a major factor in the development of neutropenia in this patient. However, the time course of the fall in wbc counts and the marrow recovery strongly suggests a link with aminoglutethimide administration. In view of this, aminoglutethimide should be used with caution in any patient likely to have a limited bone marrow reserve.

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Jeffrey Austerlitz
Internal Medicine Service
Claremore Comprehensive Indian Health Facility
Claremore, OK 74017

June 15, 1982

Intensive Timed Sequential Combination Chemotherapy in Extensive-Stage Small Cell Carcinoma of the Lung

Preliminary evaluation of an intensive timed sequential chemotherapeutic regimen in small cell carcinoma of the lung (SCCL) based on tumor cell kinetics (1) has been presented (2). The kinetic model predicts that following an initial significant tumor kill there will be stimulation of tumor growth (approximately on Days 8–11) at which time the cancer will be particularly susceptible to the effects of chemotherapy. It was our hypothesis that two cycles of this intensive induction regimen followed by local radiotherapy to the primary lesion and by maintenance chemotherapy would increase the complete response rate and prolong survival in patients with extensive-stage SCCL.

Twenty-four previously untreated patients (median age, 59 years) with extensive SCCL were treated with this protocol. A total of 54% of the patients had bone and/or bone marrow involvement with tumor while 25% had liver metastasis. A total of 66% had an Eastern Cooperative Oncology Group performance status of 0, 1, or 2. Patients were treated with CAV (75 mg/kg of cyclophosphamide iv on Day 1, 60 mg/m^2 of doxorubicin iv on Day 8, and 125 mg/m^2 of etoposide iv on Days 9–11). A second cycle of CAV was administered on Day 28. Complete and partial responders received CAVCR (25 mg/kg of cyclophosphamide iv, 40 mg/m^2 of doxorubicin iv, and 1.4 mg/m^2 of vincristine iv) on Day 56. Prophylactic cranial (2100 rads in seven fractions) and local radiotherapy to the lung primary (2000 rads in five fractions) were begun on Day 84 in an effort to decrease the incidence of brain metastasis and local recurrence. Beginning on Day 94, CAVCR was administered at 21-day intervals until relapse. Twenty-two patients were able to receive two cycles of CAV.

Eight of the 24 patients (33%) obtained a complete response (CR) and 12 (50%) obtained a partial response, for an overall response rate of 83%. Median survival for the entire group was 10.8 months, while that for patients attaining a CR was 13 months. The longest survival for patients receiving this treatment regimen was 19 months. All patients have died.

The mean duration of severe leukopenia (< 1000 wbcs/mm^3) during each induction cycle was 13 days, with the wbc count being < 500/mm^3 for a mean of 10 days. A total of 91% of the courses were associated with fever requiring antibiotics. Bacteremia developed during six courses (13%). Platelet transfusions were administered during 15 courses (33%). There were no deaths during the intensive induction therapy.

These results are comparable to our previous experience with extensive-stage SCCL (3) and that reported in the literature (4). We conclude that while intensive induction therapy with this timed sequential chemotherapeutic regimen can be safely administered, we have been unable to demonstrate a significant improvement in CR rate or survival in extensive-stage SCCL through the use of this particular regimen.

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Maurie Markman[*]
Kelly B. Pendergrass
Martin D. Abeloff

The Johns Hopkins Oncology Center
The Johns Hopkins University School of Medicine
Baltimore, MD 21205

June 21, 1982

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[*] *Reprint requests to:* Maurie Markman, MD, Division of Hematology/Oncology, University of California, H-811 K, 225 Dickinson St, San Diego, CA 92103.

Pulmonary Fibrosis Associated With Lomustine (CCNU): A Case Report

Chemotherapy with cytotoxic drugs is associated with organ-specific toxic effects. Injury to the lungs is very disturbing, since the onset of such damage is insidious. By the time damage is evident on chest X-ray or by pulmonary function studies, it may be irreversible and progressive (1). This may happen despite withdrawal of the inciting agent. Bleomycin and busulfan are well known to cause pulmonary fibrosis (1–3). Reports of lung damage have also been associated with other cytotoxic drugs.

Nitrosoureas, especially carmustine (BCNU) and lomustine (CCNU), are very effective anticancer agents and have been widely used in various solid tumors and hematologic malignancies. Delayed bone marrow suppression has been the dose-limiting toxic effect of these agents in man. Recently, more pulmonary toxicity is being described with carmustine (1,4), and there has been one report with semustine (methyl-CCNU) (5). The patient described in the current report had lung damage from lomustine while being treated for a recurrent glioma. To our knowledge, this is the first report of such damage caused by this nitrosourea.

CASE REPORT

Clinical History

A 7$^1/_2$-year-old white male was diagnosed in November 1970 as having a well-differentiated grade I astrocytoma on craniotomy and biopsy. A ventriculoperitoneal shunt was placed, and the child received radiation therapy of 2600 rads to each of three ports in the posterior cranial fossa over a 6-week period. In October 1971, the patient developed marked ataxia, inability to walk, headaches, and vomiting. A computerized tomographic (CT) scan revealed recurrence of his brain tumor, and he started receiving lomustine (130 mg/m^2) every 6 weeks orally. The total dose of lomustine was 1040 mg (1170 mg/m^2) over a period of 26 months (until December 1973). While receiving therapy, the patient had marked subjective and objective improvement in his brain tumor. In March 1972, while he was receiving lomustine therapy, he had one episode of difficulty in breathing, without fever, which cleared without treatment. There were no abnormalities of the chest X-ray at that time. There was no evidence of the tumor on CT scan at the time of discontinuation of therapy. In the first 18 months after discontinuation of therapy, there were three similar episodes of difficulty in breathing, without fever. With only one of these was a left lingular lobe infiltrate detected, which cleared within 2 weeks with antibiotics. Minimal fibrotic changes were observed on chest X-ray. In August 1975, the patient's respiratory difficulties worsened, and increased fibrosis was evident on chest X-ray at this time, but no acute infiltrates were identified. Pulmonary function studies were consistent with severe restrictive pulmonary disease. Forced vital capacity and forced expiratory volume were measured at 14% of the predicted values. Barium swallow revealed a normal esophagus, with no evidence of regurgitation. The respiratory difficulty became progressively worse, and in July 1976, on room air, PO_2 was 33 mm Hg; PCO_2, 67 mm Hg; and pH, 7.34. Chest X-ray revealed progressive fibrotic changes and a minimal right pleural effusion. No fever or other constitutional symptoms were present at this time. No evidence of brain tumor was seen on CT scan of the head. The patient's condition continued to worsen and he died within 1 week because of respiratory failure.

Autopsy Findings

An autopsy of the brain revealed a residual low-grade astrocytoma involving the pons and medulla, with men-

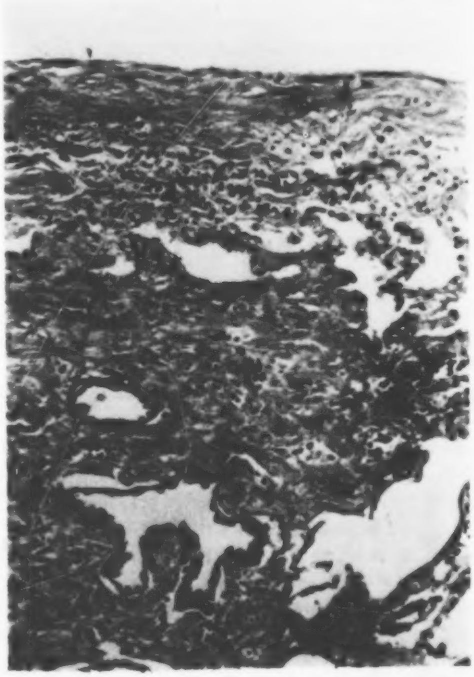

FIGURE 1.—Extensive fibrosis present subpleurally (hematoxylin and eosin stain, × 116).

ingeal spread around the cerebellum and upper cervical cord. The immediate cause of death, however, appeared to be respiratory failure. At autopsy, the right lung weighed 300 g and the left, 200 g. The surface of the right lung was covered by dense adhesions, while the left pleural surface was smooth. In both lungs, there was prominent subpleural fibrosis, most striking in the right lower lobe. The cut surface of the lungs was firmer than usual, and smaller airways showed mucous plugging. Microscopically, progressive pulmonary fibrosis was present, with lesions of different ages. In large areas, alveolar walls were thickened by fibrosis, were lined by hyperplastic and dysplastic pneumocytes, and showed septal infiltrates of a small number of mononuclear cells and an intra-alveolar accumulation of foamy macrophages, some of which contained a brown pigment. Hyaline membranes lined respiratory bronchioles and alveolar ducts in these regions. In other areas, only focal fibrotic scars were evident, obliterating bronchi and bronchioles. The pleura was thickened, and there was prominent fibrosis in the subpleural regions and in interlobular septa (fig 1). A few bronchi contained mucous plugs. In one area there was a superimposed acute bronchopneumonia. Post-mortem cultures were all negative.

DISCUSSION

The changes in the lungs resemble those seen in other examples of cytotoxic drug-induced pulmonary disease, which produce interstitial pneumonia and fibrosis. The onset in this patient was insidious, and the respiratory problems were progressive. The maximum damage occurred 2 years after discontinuation of therapy. This case emphasizes the necessity of close monitoring for lung damage from cytotoxic drugs.

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Tribhawan S. Vats
Robert C. Trueworthy

Section of Pediatric Hematology Oncology
Department of Pediatrics

Claire M. Langston

Department of Pathology

University of Kansas Medical Center
Rainbow Blvd at 39th St
Kansas City, KS 66103

July 8, 1982

Tamoxifen Before and After Orchiectomy in Advanced Male Breast Cancer

Male breast cancer is a rare disease, accounting for approximately 1% of all cases of breast cancer (1). Orchiectomy is the treatment of choice for advanced male breast cancer (2). However, it is not accepted by many patients and other modalities of hormonal treatment must be considered in these cases. The role of additive hormonal therapy has not yet been well-defined in male breast cancer. Tamoxifen, with an overall response rate of 48% in the 32 male patients reported in a review by Patterson et al (3), seems to be an effective and well-tolerated treatment. Moreover, tamoxifen seems to be more effective if employed before orchiectomy. Of nine patients with advanced male breast cancer treated with tamoxifen before orchiectomy (4–9), seven responded (4,5,7–9). Since advanced male breast cancer is too rare to be the subject of controlled studies, further reports are needed to evaluate the efficacy of tamoxifen, especially as primary treatment before orchiectomy (4). We report on four male patients with advanced breast cancer treated with tamoxifen either before or after orchiectomy. The assessment of treatment response was made according to modified International Union Against Cancer criteria (10).

CASE REPORTS

Patient 1

A 64-year-old man was first seen in August 1976 with lung and skin metastases from breast cancer (mastectomy performed in October 1979). In September 1976, orchiectomy was performed and resulted in partial remission. However, in July 1977 lung and skin metastases progressed and tamoxifen was given at a dose of 20 mg twice a day. In November 1977, skin metastases were nearly undetectable, whereas only a slight decrease of lung metastases was noted. Bone involvement became manifest in December 1977 and chemotherapy was added to tamoxifen, with progression of lung and bone metastases but with complete resolution of skin metastases. The patient died in January 1979 as a result of disseminated disease without evidence of skin lesions.

Patient 2

A 44-year-old man was first seen in December 1975 after mastectomy had been performed 1 month earlier for $T_2,N+,M_0$ breast cancer. Orchiectomy was performed as adjuvant therapy. In April 1980, bone metastases were manifested and tamoxifen was given at a dose of 20 mg twice a day, with subjective improvement until March 1981 when bone metastases progressed. The patient was then treated with chemotherapy and radiotherapy and is presently alive and continuing to receive treatment.

Patient 3

A 70-year-old man was first seen in June 1980 with a cutaneous relapse at the site of the mastectomy performed for breast cancer in September 1978. Despite the administration of tamoxifen at a dose of 20 mg twice a day, progressive disease ensued. Orchiectomy performed in August 1980 induced a complete remission lasting until January 1981. The patient is alive and receiving chemotherapy and radiotherapy.

Patient 4

A 57-year-old man was first seen in January 1981 for inoperable locally advanced breast carcinoma with axillary lymph node involvement. Tamoxifen at a dose of 20 mg twice a day was given, and there was disease progression. Orchiectomy that was performed in March 1981 induced a partial remission. Chemotherapy was then employed and the patient is still alive with his disease under control.

Tamoxifen as primary treatment was ineffective in patients 3 and 4. Subsequent orchiectomy induced objective response in these patients. On the other hand, tamoxifen was effective in patient 1, who was no longer responding to orchiectomy. This finding was also reported in the review of Patterson et al (3). Whether tamoxifen and orchiectomy are non-cross-resistant treatments in advanced male breast cancer remains to be determined in more patients and in association with estrogen-receptor determinations.

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U. Tirelli
S. Tumolo
R. Talamini
A. Veronesi
E. Galligioni
M. G. Trovo
E. Grigoletto

Division of Radiotherapy and Medical Oncology
General Hospital
33170 Pordenone, Italy

July 8, 1982

Nasal Cartilage Necrosis Following High-Dose 5-FU: A Case Report

We present a case in which necrosis of the nasal cartilage followed the use of high-dose 5-FU. As far as we know this association has not been previously reported.

In 1971, the patient, a 51-year-old woman, had a total colectomy and ileostomy for ulcerative colitis of 20 years' standing. In April 1973, an adenocarcinoma was excised from the perineum. In February 1974, her pelvis and perineum were treated with radiotherapy (5000 rads in 5 weeks, 200-rad daily fractions) for a local recurrence. She then received chemotherapy with 5-FU (500 mg iv weekly for 1 year). Following further local recurrence in April 1975 she was treated initially with iv vincristine, doxorubicin, 5-FU, methotrexate, and cyclophosphamide (five courses) and then oral cyclophosphamide, methotrexate, and razoxane until July 1978. Chemotherapy was then stopped because the woman was free of disease.

In December 1978, the patient developed further perineal recurrence confirmed histologically, and a computerized tomographic scan showed an associated pelvic mass. At the time, we were aware that the modulation of 5-FU toxicity by allopurinol had been investigated in a dose-escalation study. Stomatitis had become a dose-limiting factor at a dose of 2.25 g/m^2/day, infused over 24 hours for 5 days (1). In addition, it was then thought that allopurinol modulation might improve the therapeutic ratio. For this reason, and following a further 1600 rads to the pelvis, she was given 2.8 g (2 g/m^2/day) of 5-FU as an infusion for 5 consecutive days. Allopurinol (300 mg three times a day) was given concurrently and for 48 hours before the 5-FU. The day after the course was completed the patient noticed stuffiness of the nose associated with gradual swelling. Following this she developed pancytopenia and severe oral mucositis necessitating iv feeding. As her oral symptoms improved, the patient noticed what she thought to be increasing prominence of the dorsal bony nasal hump. On examination, however, it became evident that this was due to gradually increasing saddling of the cartilaginous dorsum, associated with lack of nasal septal cartilage support. Anterior rhinoscopy showed that the mucous membranes of the septum were intact but that the cartilage had absorbed as would be the case following an extensive submucous resection. There was no past history, however, of any nasal surgery or trauma. X-rays of the sinuses and the nasal bones revealed no evidence of residual cartilage, and biopsy of the mucous membranes might have led to a troublesome septal perforation.

The patient was then treated with 5-FU in conventional doses, but after achieving a partial response for 1 year, she died following disease progression.

DISCUSSION

There is no doubt clinically that this patient developed necrosis of her nasal septal cartilage. Known causes of nasal cartilage necrosis such as trauma, syphilis, or cocaine abuse have been excluded. The fact that the event was immediately preceded by high-dose 5-FU suggests a causative link between the two. There have been no previous reports of nasal septal cartilage necrosis with either 5-FU or allopurinol, and neither drug is concentrated in cartilage (2). It is possible that the patient developed nasal mucosal edema as part of her generalized mucositis and that this led to compression of the perichondrium and to avascular necrosis.

We have now treated a total of 34 patients with high-dose 5-FU and allopurinol modulation, using the lower dose of 5-FU (1.5 g/m^2), infused over 6 hrs. This is the subject of a separate report (3). We have had no other cases of nasal cartilage necrosis, although there were three cases of pancytopenia and three cases of mucositis. Unfortunately, there was no clinical benefit.

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R. F .U. Ashford
T. Mughal
M. A. Goold
B. J. Smith
R. H. Phillips

Stanford Cade Unit of Clinical Oncology
Westminster Hospital
Dean Ryle St-Horseferry Rd
London SWIP 2AP, England

I. S. MacKay

St. Stephens Hospital
Fulham Rd
London SW10, England

July 14, 1982

Adverse Reactions to AMSA in Medical Personnel

We have observed unusual reactions to AMSA in nine pharmacy and nursing personnel who have been involved in mixing the drug in preparation for administration.

A 21-year-old pharmacy technician developed an urticarial rash on the arms and trunk within minutes after mixing AMSA in a horizontal vented hood within the hospital pharmacy. He was given 50 mg of diphenhydramine hydrochloride orally and the rash subsided within 4 hours. Two days later, although he wore surgical gloves, the same reaction occurred following the preparation of the drug. When he prepared the drug 5 days later, wearing gloves, surgical mask, and surgical gown, the reaction developed for a third time. No other symptoms were noted. The pharmacist, a 30-year-old white female, relieved the technician of this responsibility and prepared the drug solution herself 2 days later. While adding the diluent, the vial shattered and some of the drug spilled on her hands and clothing. She immediately washed the drug from her hands but did not change clothes. Four hours later she developed nausea followed by vomiting. Except for light-headedness, she had no other symptoms. The nausea and vomiting abated after 4 hours and she felt well the next morning.

Several weeks earlier, six of eight pharmacy technicians and nurses responsible for the handling of AMSA in another hospital had experienced unusual symptoms within days of each other. In each instance the symptoms developed after handling AMSA. The symptoms began between 5 minutes and 3 hours after mixing the drug and included nausea, light-headedness, headache, sharp epigastric pain, and malaise. The symptoms in this group of persons lasted from 1 to 12 hours. One technician subsequently developed similar symptoms while standing adjacent to the area where the drug was being mixed but did not develop symptoms when he mixed the drug a few days later using a vertical vented hood.

A few weeks later, a pharmacy technician at a third hospital developed similar symptoms of nausea, light-headedness, malaise, and headache after mixing AMSA.

These apparent drug reactions prompted the recommendation that a vertical vented hood be used for mixing of the drug. Horizontal vented hoods were not recommended because the direction of airflow would result in potential direct drug exposure as the drug is being prepared. No additional reactions have been reported in pharmacy or nursing personnel after 3 months of observation following the recommendation that a vertical hood be used.

In summary, nine pharmacy and nursing personnel have developed symptoms compatible to the toxic effects of AMSA. Seven of the personnel have developed nausea, light-headedness, headache, and malaise within hours after mixing the drug. One person developed nausea and one developed an urticarial rash on three separate occasions after mixing AMSA. Additional reactions appear to have been prevented by mixing the drug in a vertical vented hood in the pharmacy.

Ralph D. Reynolds[1,*]
Robert Ignoffo[2]
Jeffrey Lawrence[3]
Frank M. Torti[4]
Maria Koretz[4]
Nancy Anson[1]
Ardis Meier[1]

July 21, 1982

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[1] David Grant USAF Medical Center, Travis Air Force Base, CA.

[2] Northern California Oncology Group Pharmacy Committee, San Francisco.

[3] University of California Davis School of Medicine, Sacramento.

[4] Northern California Oncology Group, Palo Alto.

*_Reprint requests to:_ Dr. Ralph D. Reynolds, Hematology Oncology Service, Department of the Air Force, David Grant USAF Medical Center (MAC), Travis Air Force Base, CA 94535.

ANNOUNCEMENTS

Fifth Annual San Antonio Breast Cancer Symposium

San Antonio, Texas, November 5–6, 1982

The program of scientific presentations will be complemented by state of the art lectures by invited speakers: Anthony B. Miller, National Cancer Institute of Canada, "Screening Breast Cancer"; William L. McGuire, The University of Texas Health Science Center at San Antonio, "New Approaches to Understanding the Biological Behavior of Breast Cancer"; Marvin Zelen, Harvard University School of Public Health, "The Innovative Approaches to the Design of Clinical Trials in Breast Cancer"; and James H. Goldie, Cancer Control Agency of British Columbia, "Chemotherapeutic Strategy Based Upon Mutation to Resistance in Breast Cancer."

Symposium coordinators are Drs. William L. McGuire and Charles A. Coltman, Jr.

For further information, contact: Terri McDaniel, RN, Cancer Therapy and Research Center, 4450 Medical Dr., San Antonio, TX 78229 (telephone: 512-690-0655).

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Treatment of Advanced Gastrointestinal Cancer

Padova, Italy, June 23–24, 1983

The aim of this symposium, sponsored by the European Organization for Research on Treatment of Cancer, is to evaluate the current modalities available for the treatment of advanced tumors of the gastrointestinal tract. The meeting will focus on chemotherapy, radiotherapy, and surgery, either alone or in combination. The program will include invited presentations and free communications. Free communications will be selected on the basis of submitted abstracts. Abstracts should be received by March 31, 1983, and addressed to Pr. M. Lise, Director, Istituto di Patologia Chirurgica I°, Universita degli Studi di Padova, 64 via Gattamelata, 35100 Padova, Italy.

Seating is limited and preregistration is recommended. The registration fee is $90.

For further information, contact: Ms. D. Eeckhoudt, Executive Secretary, EORTC Data Center, 1 rue Héger-Bordet, 1000 Brussels, Belgium (telephone: (2) 539.30.20 - Telex: 22773).

Two International Conferences on Cancer Treatment

Zürich, Switzerland, July 18–23, 1983

The Division of Oncology at the Universitätsspital Zürich is hosting two international conferences titled "Present Status of Interferon in Clinical Cancer Research" and "Recent Developments of In Vitro Tests for Predicting Response to Anti-Cancer Treatment." Due to the current importance and recent progress in these two subjects, a large number of active participants are expected from around the world.

Among the 20 guest speakers that will take part are: Drs. Lindenmann and Weissmann (Zurich); Salmon and Durie (USA); Steel and Courtenay (UK); and Silvestrini (Italy). The program for these meetings will be ready by September 1982.

For further information, contact: Dr. V. Hofmann, Universitätsspital Zürich, Department of Internal Medicine, Division of Oncology, 8091 Zürich, Ramistrasse 100, (telephone 01 2552214).

Clinical Trials in Cancer Medicine: Past Achievements and Future Prospects

Venice, Italy, October 19–21, 1983

The sixth International Bristol-Myers Symposium will be organized by Drs. Umberto Veronesi and Gianni Bonadonna of the Istituto Nazionale Tumori, Milan, Italy, and will take place at the Fondazione Cini. The meeting will be directed to surgical, medical, and pediatric oncologists, radiation therapists, and medical statisticians. The format of the symposium will include a series of presentations followed by discussion. Known cancer specialists from the United States and Europe will critically review the design and the results of major therapeutic trials performed during the past two decades in various neoplastic diseases of adults and children. All formal presentations will be collected in a book edited by U. Veronesi and G. Bonadonna, and will be published in 1984 by Academic Press, New York.

English will be the official language at the symposium and no simultaneous translation will be provided. The registration fee is $150 and lunch will be provided at the Fondazione Cini on October 20 and 21. Only the first 550 applicants who return their registration and hotel forms and pay the fee before March 31, 1983, will be accepted.

For further information, contact: Mr. Renzo Polacco, Trident, Gran Viale 2, Lido di Venezia 30126, Italy (telephone: 041-767.188 or 760.555 - Telex: 410120), before December 31, 1982.

New Drugs in Cancer Therapy

Brussels, Belgium, December 15–17, 1983

The Fourth European Organization for Research on Treatment of Cancer-National Cancer Institute (EORTC-NCI) Symposium will focus on phase I and phase II clinical data including drug pharmacology with investigational anticancer agents. Preclinical aspects in the new drug development area will also be covered, with particular emphasis on the role of the human stem cell assay in the screening of new antitumor drugs. The program will include invited presentations and free communications. Free communications will be selected on the basis of submitted abstracts. Abstracts must be received at the EORTC Data Center by October 31, 1983. Seating is limited and preregistration is recommended. The registration fee is $135.

For further information, contact: Dr. M. Rozencweig or Dr. M. Staquet, EORTC Data Center, 1 rue Héger-Bordet, 1000 Brussels, Belgium (telephone: (2) 539.30.20., telex: 22773).

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a publication
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NIH Publication No. 83-22

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cancer treatment reports

November 1982
Volume 66
Number 11
Pages 1889-2004

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

HE 20.3160:

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The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1984.

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1939 Toxicology of AMSA in Beagle Dogs and CDF₁ Mice. **K. L. Pavkov and D. C. Thake.** Battelle Columbus Laboratories, Ohio

1945 Activity of 1-*p*-(3,3-Dimethyl-1-triazeno) Benzoic Acid Potassium Salt in M 5076/73A Ovarian Reticular Cell Sarcoma of the Mouse. **T. Colombo, S. Garattini, L. Lassiani, and M. D'Incalci.** Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy

1949 Hematopoietic Stem Cells in Friend Murine Leukemia Virus-Infected Mice Undergoing Chemotherapy: Effects of 5-FU. **H. J. Seidel and L. Kreja.** University of Ulm, W Germany

1957 Studies of the Pharmacology of *N*-Methylformamide in Mice. **C. Brindley, A. Gescher, E. S. Harpur, D. Ross, J. A. Slack, M. D. Threadgill, and H. Whitby.** CRC Experimental Chemotherapy Research Group, University of Aston, Birmingham, England

BRIEF REPORTS

1967 Massive Vincristine Overdose: Failure of Leucovorin To Reduce Toxicity. **L. T. Thomas, P. C. Braat, R. Somers, and R. Goudsmit.** University Hospital, Whilhelmina Gasthuis, University of Amsterdam, The Netherlands

1971 Combination Chemotherapy for Multiple Myeloma With Melphalan, Prednisone, Cyclophosphamide, Vincristine, and Carmustine (BCNU) (M-2 Protocol). **U. Tirelli, D. Crivellari, A. Carbone, A.**

Veronesi, E. Galligioni, M. G. Trovò, S. Tumolo, and E. Grigoletto. General Hospital, Pordenone, Italy

1975 Results of a Randomized Double-Blind Crossover Trial of Scopolamine Versus Placebo Administered by Transdermal Patch for the Control of Cisplatin-Induced Emesis. **D. L. Longo, M. Wesley, D. Howser, S. M. Hubbard, T. Anderson, and R. C. Young.** National Cancer Institute, Bethesda, Maryland

1977 Salvage Chemotherapy for Non-Hodgkin's Lymphoma. **R. P. Ng, D. Todd, and R. K. K. Khoo.** Queen Mary Hospital, Hong Kong

1981 Ip Immunotherapy and Chemotherapy in Advanced Epithelial Ovarian Cancer. **E. Hernandez, N. B. Rosenshein, J. Pino y Torres, J. Villar, F. C. Grumbine, D. S. Ettinger, M. B. Dillon, J. L. Klein, and S. E. Order.** The Johns Hopkins Medical Institutions, Baltimore, Maryland

1983 Autologous Bone Marrow Transplantation in Acute Myeloid Leukemia in Relapse or in Complete Remission. **P. Herve, A. Rozenbaum, E. Plouvier, M. Flesch, J. Y. Cahn, A. Farradji, J. P. Bergerat, J. C. Adjizian, and B. Pignon.** Centre Régional de Transfusion Sanguine, Besançon, France

1987 Multiple Squamous Cell Carcinoma Treated With Intra-arterial Cisplatin in a Patient With Rheumatoid Arthritis. **J. A. Ajani and M. A. Burgess.** University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

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1993 *m*-AMSA in Disseminated Prostatic Carcinoma: A Phase II Study. **A. Drelichman, D. A. Decker, M. Al-Sarraf, and C. B. Dhabuwala.** Wayne State University, School of Medicine, Detroit, Michigan

1995 Phase II Trial of Mitoxantrone in Advanced Colorectal Cancer. **E. M. Bonnem, E. P. Mitchell, P. V. Woolley, F. P. Smith, J. Neefe, L. Smith, and P. S. Schein.** Georgetown University Hospital and Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC

1997 Phase II Trial of Bisantrene in Advanced Colorectal Cancer: A Cancer and Leukemia Group B Study. **M. C. Perry, A. A. Forastiere, F. Richards, II, R. B. Weiss, and D. Anbar.** Cancer and Leukemia Group B

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2000 Subcutaneous Reaction Due to Intra-arterial Infusion of Doxorubicin. **A. Ballin, G. Kende, B. Morag, and M. Ronen.** Chaim Sheba Medical Center, Tel-Hashomer, Israel

2003 ANNOUNCEMENTS

Influence of Diuretics on Urinary General Base Catalytic Activity and Cyclophosphamide-Induced Bladder Toxicity[1,2,3]

N. E. Sladek,* P. C. Smith, P. M. Bratt, J. E. Low, J. F. Powers, R. F. Borch, and J. R. Coveney[4]

The influence of diuretics on the induction of bladder toxicity by cyclophosphamide was investigated in rats. Following ip administration, about 3.5% of the cyclophosphamide was excreted as 4-hydroxycyclophosphamide. This amount was found to be compatible with the view that the urotoxic effects of cyclophosphamide are caused by the acrolein generated in the urine from 4-hydroxycyclophosphamide, the primary metabolite of cyclophosphamide. In situ, acrolein was more potent than 4-hydroperoxycyclophosphamide with regard to producing an increase in bladder weight; phosphoramide mustard was essentially without urotoxic activity. The urotoxic potency of 4-hydroperoxycyclophosphamide, but not that of acrolein, increased as the pH and/or the phosphate concentration of the infusion medium increased. This was as expected in view of the knowledge that release of acrolein from 4-hydroxycyclophosphamide or 4-hydroperoxycyclophosphamide is facilitated by the presence of general base catalysts, eg, phosphate and bicarbonate, and that the rate at which this reaction proceeds in the presence of these catalysts increases as their concentration and the pH increases. In vivo, diuretics that acidified the urine, eg, ammonium chloride and furosemide, prevented the increase in bladder weight ordinarily elicited by the dose of cyclophosphamide used in these experiments. In contrast, a diuretic, acetazolamide, that markedly increased urinary bicarbonate concentration and alkalinized the urine, did not. None of the diuretics altered the systemic metabolism and urinary excretion of cyclophosphamide nor did they alter the systemic action, as judged by spleen weight, of cyclophosphamide. These observations demonstrate that the pH of the urine and the urinary concentration of general base catalysts greatly influence the urotoxic potential of oxazaphosphorines such as cyclophosphamide. They indicate that while the use of acidifying diuretics is likely to be beneficial in minimizing oxazaphosphorine-induced bladder toxicity, the use of alkalinizing diuretics may not be helpful. [Cancer Treat Rep 66:1889–1900, 1982]

The clinical usefulness of the oxazaphosphorines cyclophosphamide and ifosfamide is frequently limited by their urotoxic effects, ie, hemorrhagic cystitis. This toxicity is effected largely by the acrolein released in the urine from 4-hydroxycyclophosphamide and 4-hydroxyifosfamide, the primary metabolites of cyclophosphamide and ifosfamide, respectively (1-3). Release of acrolein is facilitated by the presence of general base catalysts, eg, phosphate and bicarbonate; the rate of release increases as their concentration and/or pH increases [(4,5); J. E. Low and N. E. Sladek, unpublished observations].[5]

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[1] Received Feb 8, 1982; revised June 3, 1982; accepted June 16, 1982.

[2] Supported by Public Health Service grant CA-21737 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] This is the 11th in a series of papers on cyclophosphamide metabolism. Parts of this investigation appear in abstract form (Pharmacologist 22:175, 1980).

[4] Department of Pharmacology, University of Minnesota, Minneapolis.

[5] In previous communications (4,5), we interpreted our data as indicating that the mechanism by which phosphate (and bicarbonate) facilitated the conversion of 4-hydroxycyclophosphamide to acrolein and phosphoramide mustard was one of bifunctional catalysis. Recent, as yet unpublished, findings in our laboratory challenge this view and indicate that the mechanism is one of general base catalysis. Therefore, the term "general base catalysts" is used in this paper to refer to those agents, eg, phosphate and bicarbonate, that catalyze the conversion of 4-hydroxycyclophosphamide to acrolein and phosphoramide mustard.

**Reprint requests to.* Dr. N. E. Sladek, Department of Pharmacology, University of Minnesota, 3-260 Millard Hall, 435 Delaware St SE, Minneapolis, MN 55455.

Diuretics are often used to mitigate the urotoxic effects of these drugs. Their use is based on the rationale that they will effectively cause a dilution of the toxic material and decrease its contact time with the bladder. Some diuretics affect urinary bicarbonate and phosphate content; the pH of the urine may also be changed.

Little is known about the effects of diuretics on the systemic and extrasystemic actions of the oxazaphosphorines. The effects of several diuretics on cyclophosphamide metabolism and cyclophosphamide-induced increased bladder weight and spleen atrophy were determined in this investigation. Also determined were the urinary excretion of 4-hydroxycyclophosphamide following the ip administration of cyclophosphamide and the influence of urinary pH and general base catalyst concentration on the urotoxic potential of cyclophosphamide metabolites in situ.

MATERIALS AND METHODS

Animals.—Sprague-Dawley rats were purchased from the Bio-Lab Corp, White Bear Lake, MN. They were housed in metabolic cages and were fed Purina Laboratory Chow (Ralston Purina Co, St. Louis) ad libitum except during the 6-hr urine collection period when all food was removed. Water was available throughout, except where indicated in specific experiments.

Drugs. — Cyclophosphamide, 4-hydroperoxycyclophosphamide, and phosphoramide mustard· cyclohexylamine were supplied by Dr. W. A. Zygmunt, Mead Johnson and Co, Evansville, IN; Dr. Richard Borch, University of Minnesota, Minneapolis; and Dr. H. B. Wood, Jr, Drug Development Branch, 2ivision of Cancer Treatment, National Cancer Institute, Bethesda, MD; respectively. Furosemide (Lasix) and acetazolamide sodium (Diamox) were purchased from the University of Minnesota Hospital Pharmacy, Minneapolis. Acrolein and ammonium chloride were purchased from the Aldrich Chemical Co, Milwaukee; and Matheson, Coleman & Bell Manufacturing Chemists, Norwood, OH; respectively.

Drug administration.—Cyclophosphamide was dissolved in 0.9% NaCl solution and was injected ip in a volume equivalent to 3 ml/kg. Acetazolamide, ammonium chloride, and furosemide were dissolved in water and were given orally in a volume equivalent to 8.8 ml/kg. Doses and administration schedules are noted with each experiment. All of the drug solutions were administered immediately after preparation.

Cyclophosphamide assay.—The cyclophosphamide content of blood and urine obtained from treated rats was determined by a gas chromatography method described previously (6). Blood samples (140 µl) were collected from the tail vein into heparinized pipettes at 15, 30, 45, 60, 90, 120, and 150 mins when the systemic half-life of cyclophosphamide was determined. Values obtained at 30–120 mins inclusive were used to calculate the half-life of cyclophosphamide.

Assay for plasma 4-hydroxycyclophosphamide.—A fluorometric assay (7,8) was used to determine plasma concentrations of 4-hydroxycyclophosphamide. Blood samples (140 µl) were collected from the tail vein into heparinized pipettes. Following centrifugation, 50 µl of the resultant plasma was placed into a tube containing 25 µl of an aqueous 10% $Na_2WO_4 \cdot 2H_2O$ solution and 200 µl of an aqueous 0.01 N HCl solution. The contents were vortexed, 250 µl of an aqueous 12% $HC10_4$ solution was added, and the contents were vortexed again. Following centrifugation, 350 µl of the resultant supernatant was added to 200 µl of *m*-aminophenol (5 mg/ml) and hydroxylamine hydrochloride (6 mg/ml) dissolved in aqueous 3 N HCl solution. All of the previous steps were performed at approximately 4°C. The reaction mixture was then placed into a boiling water bath for 15 mins, after which it was cooled to room temperature in the dark. The concentration of the product, 7-hydroxyquinoline, was determined in an Aminco-Bowman J4-8950 spectrophotofluorometer. The excitation wavelength was 346 nm; the emission wavelength was 500 nm. Plasma samples, taken before cyclophosphamide was administered, were used to obtain blank values. 4-Hydroperoxycyclophosphamide was used as the standard. The lower limit of sensitivity was approximately 1 nmol of 4-hydroperoxycyclophosphamide/ml of plasma. The assay is relatively specific for acrolein and compounds (eg, 4-hydroperoxycyclophosphamide, 4-hydroxycyclophosphamide, and aldophosphamide) that yield acrolein. However, although acrolein placed in water gave rise to net relative fluorescent intensities comparable to those effected by equivalent concentrations of 4-hydroperoxycyclophosphamide, acrolein placed in blood gave rise to net relative fluorescent intensities that were < 1/100 of those obtained when equivalent concentrations of 4-hydroperoxycyclophosphamide were placed in blood; the disparity was apparently due to the highly reactive nature of acrolein. The presence of aldophosphamide in biologic fluids has never been conclusively demonstrated, probably because of its very short half-life (5,9).

Assay for urinary 4-hydroxycyclophosphamide.—Male Sprague-Dawley rats weighing 300–350 g were anesthetized with 100 mg/kg of Inactin ip. Following the start of anesthesia, the right jugular vein and the ureters were catheterized with PE-50 and PE-10 polyethylene tubing, respectively. Normal saline solution was infused into the jugular vein at a rate of 55 µl/min throughout the remainder of the experiment to ensure a constant urine flow of 11 ± 1 µl/min. Two hrs after the initiation of the normal saline infusion, 50 mg/kg of cyclophosphamide in normal saline solution was given iv in 0.5 ml over a 5-min period. Collection of the urine was initiated 1 hr after the start of the normal saline infusion. It was collected for ten successive 30-min periods into tubes containing 10 ml of water; the tubes were in ice water throughout the collection period. Preliminary experiments established the stability of 4-hydroxycyclophosphamide for 30 mins under these conditions. The fluorometric assay described above was then used to determine urinary concentrations of 4-hydroxycyclophosphamide. As above, 50-μl samples were used for this purpose. Urine collected during the 1-hr period immediately before the injection of cyclophosphamide was used to obtain blank values; preliminary experiments established that these did not vary appreciably during the collection period. Authentic 4-hydroperoxycyclophosphamide was used as the standard. The lower limit of sensitivity was approximately 1 nmol of 4-hydroperoxycyclophosphamide/ml of urine.

Bladder infusion and assessment of urotoxicity.—Female Sprague-Dawley rats weighing 155–200 g were anesthetized with either 30 mg/kg of pentobarbital ip or pentobarbital and ketamine, each given ip at a dose of 15 mg/kg. Water was removed at least 12 hrs before initiation of anesthesia to minimize urine production. Following the start of anesthesia, PE-50 polyethylene tubing was passed into the bladder via the urethra, the bladder was flushed twice with 0.2 ml of normal saline solution, and the catheter was removed. A second catheter, the void volume of which was cleared with the appropriate drug solution, was passed into the bladder and secured with 3-0 silk thread. Acrolein, phosphoramide mustard, or 4-hydroperoxycyclophosphamide in sodium phosphate buffer solution was then infused into the bladder and allowed to remain for 1 hr. The infusion volume was 0.2 ml. At the end of this period, the drug was removed, the bladder was flushed twice with 0.2 ml of normal saline solution, and the catheter was removed. Bladders were removed 48 hrs later, cleaned of their contents, blotted dry, and weighed.

Other assays.—Bicarbonate and inorganic phosphate concentrations were determined by methods described previously (10,11).

Data analysis.—Values were submitted to computer-assisted linear regression analysis to obtain log-linear functions.

RESULTS

Cyclophosphamide-induced bladder toxicity is reflected by an increase in bladder weight although bladder wet weight can be used as a valid criterion of the urotoxic potency of the oxazaphosphorines only within a limited dose range (3). The relationship between dose and response is shown in figure 1. As presented, the data suggest that male rats are more sensitive to the urotoxic action of cyclophosphamide than are female rats. However, when bladder weights are expressed per total body weight, the apparent sex difference becomes less obvious (calculations not presented). The systemic effects of cyclophosphamide include a decrease in spleen weight. The relationship between dose and response is shown in figure 2.

Furosemide and NH_4Cl effected a diuresis, acidified the urine, and prevented the increase in bladder weight usually caused by the dose of cyclophosphamide used; the urinary concentrations of bicarbonate and phosphate were decreased or unchanged (table 1). In contrast, acetazolamide, an alkalinizing diuretic, did not prevent the cyclophosphamide-induced increase in bladder weight even though it effected a greater diuresis than did NH_4Cl; the phosphate concentration was slightly decreased and the bicarbonate concentration was markedly increased. Furosemide and NH_4Cl also prevented the gross hematuria induced by 100 mg/kg of cyclophosphamide; acetazolamide did not (data not presented). Ammonium acetate, at a dose equimolar to that of NH_4Cl, did not prevent cyclophosphamide-induced bladder toxicity nor did it alter urine pH and flow (data not presented). None of the diuretics altered the systemic action of cyclophosphamide as judged by their inability to prevent the loss of spleen weight (table 1).

Plasma concentrations of 4-hydroxycylophosphamide following the administration of 50 mg/kg of cyclophosphamide to control rats are presented in figure 3. 4-Hydroxycyclophosphamide is cleared from the plasma with an apparent half-life of 37 mins. The half-life for cyclophosphamide was virtually identical (table 2).

Consistent with the lack of an effect by any of the diuretics on the systemic action of cyclophosphamide are the observations that they did not alter the constants that define the metabolism, distribution, and excretion of cyclophosphamide and 4-hydroxycyclophosphamide (table 2).

The next series of experiments was conducted to establish that the concentration of general base catalysts in, and the pH of, the urine are important determinants with regard to the potential of cyclophosphamide to induce hemorrhagic cystitis and to offer an explanation for the inability of acetazolamide to reduce the bladder toxicity associated with cyclophosphamide administration even though urine flow is increased. Inorganic phosphate rather than bicarbonate was chosen as the general base catalyst to illustrate these points because of the relative ease with which its concentration can be maintained at a fixed value over a prolonged period of time.

Infusing bladders with acrolein effected a dose-dependent increase in bladder weight (fig 4). Bladder weights of 178 mg were obtained when 0.38 μmol of acrolein (1.9 mM) was infused. Similar observations have been reported (1,3). Administration of 50 and 90 mg/kg of cyclophosphamide ip to male (230–260 g) and female (170–200 g) rats, respectively, caused an identical increase in bladder weight (fig 1). The rate at which ad-

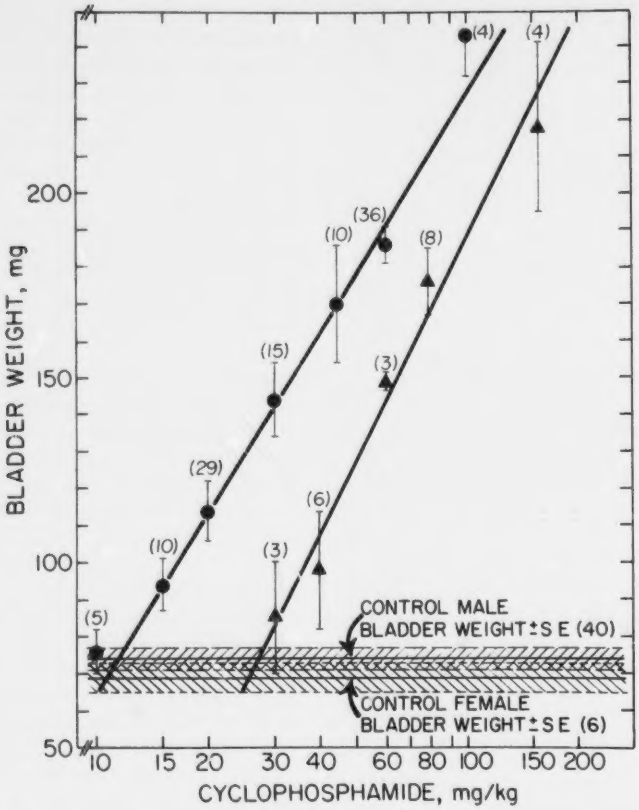

FIGURE 1.—Bladder weights in cyclophosphamide-treated rats: dose-response relationship. Male (●) and female (▲) Sprague-Dawley rats, 230–260 and 170–200 g, respectively, were injected ip with the doses of cyclophosphamide indicated. Bladder wet weights were determined 48 hrs later. Values are the mean ± SE; No. of animals is shown in parentheses.

TABLE 1.—Urinary and tissue weight changes in rats treated with cyclophosphamide and/or diuretics*

Treatment	Cyclophosphamide	Urine				Tissue weight (mg)	
		pH	Volume (ml)	PO_4 (mM)	HCO_3 (mEq/L)	Bladder	Spleen
H_2O	–	6.8 ± 0.1	3.4 ± 0.6	34 ± 5	7 ± 3	74 ± 3	572 ± 20
Acetazolamide	–	8.2 ± 0.2	10.6 ± 0.9	12 ± 2	133 ± 5	89 ± 7	578 ± 31
NH_4Cl	–	5.9 ± 0.1	7.3 ± 0.7	25 ± 4	1 ± 1	72 ± 5	684 ± 63
Furosemide	–	6.0 ± 0.1	18.7 ± 0.7	10 ± 2	3 ± 1	71 ± 3	545 ± 34
H_2O	+	6.8 ± 0.3	3.7 ± 0.7	32 ± 6	3 ± 1	186 ± 5	327 ± 12
Acetazolamide	+	8.1 ± 0.2	11.6 ± 0.8	14 ± 1	115 ± 4	168 ± 15	325 ± 11
NH_4Cl	+	5.8 ± 0.1	5.5 ± 0.2	30 ± 4	4 ± 1	79 ± 3	417 ± 13
Furosemide	+	6.1 ± 0.1	16.9 ± 5.2	14 ± 1	1 ± 1	93 ± 6	338 ± 30

*Male Sprague-Dawley rats, 230–260 g, were treated with the following diuretics before and/or after cyclophosphamide or 0.9% NaCl administration: 10 mg/kg of acetazolamide orally 15 mins before and 105 mins after, 660 mg/kg of NH_4Cl orally 120 mins before, or 88 mg/kg of furosemide orally 15 mins before. All diuretics were given as aqueous solutions; the volume given each time was 8.8 ml/kg. Animals that did not receive diuretics were given identical volumes of water. Values obtained for the 2 "H_2O" groups were pooled in each case because they were essentially identical. Cyclophosphamide was dissolved in 0.9% NaCl solution and 60 mg/kg was injected ip; the volume injected was 3 ml/kg. Animals that did not receive cyclophosphamide were injected with identical volumes of 0.9% NaCl solution. Urine was collected during the 6-hr period following cyclophosphamide injection; volume and pH of these collections were measured as were inorganic phosphate and bicarbonate concentrations. Bladder and spleen wet weights were determined 48 hrs after injection of cyclophosphamide. Values are the mean ± SE of measurements made on 3–18 animals.

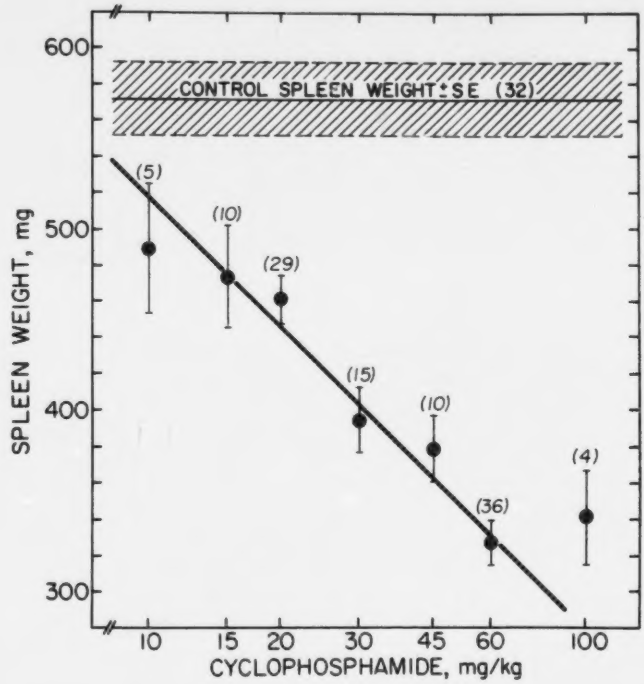

FIGURE 2.—Spleen weights in cyclophosphamide-treated rats: dose-response relationship. Male Sprague-Dawley rats, 230–260 g, were injected ip with the doses of cyclophosphamide indicated. Spleen wet weights were determined 48 hrs later. Values are the mean ± SE; No. of animals is shown in parentheses.

ducts between acrolein and sulfhydryls (ie, glutathione, acetylcysteine, or cysteine) are generated increases as the pH increases (12), suggesting the possibility of a similar relationship between acrolein and the critical target sites of the bladder. However, the increase in bladder weight effected by acrolein was essentially independent of the pH and phosphate concentration of the infusion medium (fig 4).

Infusing bladders with 4-hydroperoxycyclophosphamide also effected a dose-dependent increase in bladder weight (fig 5). 4-Hydroperoxycyclophosphamide was used in these experiments rather than 4-hy-

TABLE 2.—Half-lives of cyclophosphamide and 4-hydroxycyclophosphamide in rats treated with diuretics*

	Cyclophosphamide		4-Hydroxycyclophosphamide		
Diuretic	Half-life (mins)	% excreted in urine	Apparent half-life (mins)	Peak concentration (μM)	Area under curve (mM·min)
None	37 ± 4	8.3 ± 1.0	37 ± 6	27.6 ± 0.4	1.9 ± 0.1
Acetazolamide	35 ± 1	9.9 ± 0.5	42 ± 4	30.8 ± 2.1	2.0 ± 0.1
NH_4Cl	39 ± 4	6.9 ± 0.8	36 ± 4	30.5 ± 4.9	1.9 ± 0.2
Furosemide	33 ± 2	9.4 ± 1.9	44 ± 7	28.8 ± 1.6	1.9 ± 0.2

*Male Sprague-Dawley rats, 230–260 g, were injected ip with cyclophosphamide dissolved in a 0.9% NaCl solution; the volume injected was 3 ml/kg and doses were 100 and 50 mg/kg when concentrations of cyclophosphamide and 4-hydroxycyclophosphamide, respectively, were determined. Animals were also given 10 mg/kg of acetazolamide orally 15 mins before and 105 mins after cyclophosphamide, 660 mg/kg of NH_4Cl orally 120 mins before cyclophosphamide, or 88 mg/kg of furosemide orally 15 mins before cyclophosphamide. All diuretics were given as aqueous solutions; the volume given each time was 8.8 ml/kg. Control animals received identical volumes of water; values obtained for these animals were pooled because they were essentially identical. Half-lives were determined as described in fig 3 and Materials and Methods section. Urine was collected during the 24-hr period following cyclophosphamide injection; cyclophosphamide content of these collections was measured. Of the total cyclophosphamide excreted in the urine, > 90% was excreted in the first 12 hrs following cyclophosphamide administration. Values are the mean ± SE of measurements made on 3–10 animals.

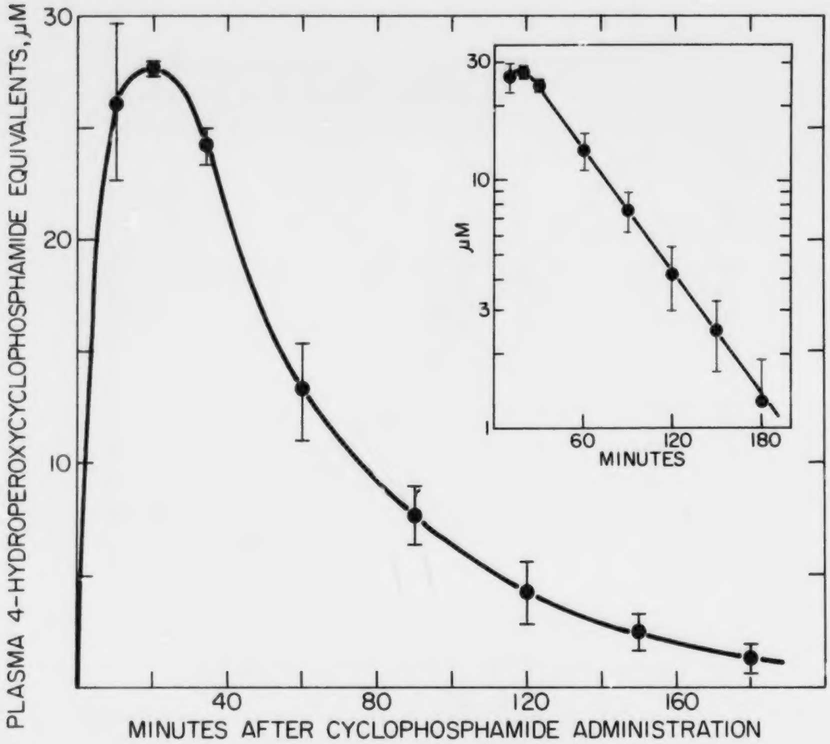

FIGURE 3.—Plasma concentrations of 4-hydroxycyclophosphamide following the administration of cyclophosphamide to rats. Male Sprague-Dawley rats, 230–260 g, were injected ip with 50 mg/kg of cyclophosphamide, and plasma concentrations of 4-hydroxycyclophosphamide were determined as described in Materials and Methods section. Values are the mean ± SE of measurements made on 5 animals. A semilog plot of the data is presented in the inset; the apparent half-life was 37 mins. The 30–150-min values, inclusive, were used to generate the apparent plasma half-lives presented in table 2 for 4-hydroxycyclophosphamide.

droxycyclophosphamide because the former readily gives rise to the latter in aqueous phosphate solution (5,13) and because 4-hydroxycyclophosphamide is relatively much less stable, making it more difficult to work with. Conversion of 4-hydroperoxycyclophosphamide to 4-hydroxycyclophosphamide does not appear to require the presence of a general base catalyst (5). Bladder weights of 178 mg were obtained when 3.8 µmols of 4-hydroperoxycyclophosphamide (19 mM) was infused, the pH was 8, and the phosphate concentration was 40 mM. Brock et al (1,3) obtained similar results using 4-hydroxycyclophosphamide. That 4-hydroperoxycyclophosphamide was not as potent as acrolein was expected, given that 4-hydroperoxycyclophosphamide must give rise to acrolein before toxicity is effected. Conversion of 4-hydroperoxycyclophosphamide to 4-hydroxycyclophosphamide in similar media takes several minutes (5,13) and the half-life of 4-hydroxycyclophosphamide in 40 mM phosphate buffer at pH 8 is about 70 mins (5). The increase in bladder weight induced by 4-hydroperoxycyclophosphamide was, as expected, dependent on the pH and the phosphate concentration of the medium; decreasing the pH or the phosphate concentration measurably decreased the potency of 4-hydroperoxycyclophosphamide (fig 5). For example, at pH 6 and a phosphate concentration of 4 mM, 20 mM 4-hydroperoxycyclophosphamide caused little or no increase in bladder weight above control; the half-life of 4-hydroxycyclophosphamide in 4 mM phosphate buffer at pH 6 is about 2000 mins (5). Brock et al (3) used 4-hydroxycyclophosphamide and observed similar pH dependency. The effects of higher concentrations of 4-hydroperoxycyclophosphamide could not be determined according to the protocol used because of solubility problems.

Phosphoramide mustard, even at the relatively high concentration of 50 mM (10 µmols), was essentially without effect with regard to increasing the bladder weight, regardless of the pH or the phosphate concentration of the infusion medium. However, there was a statistically

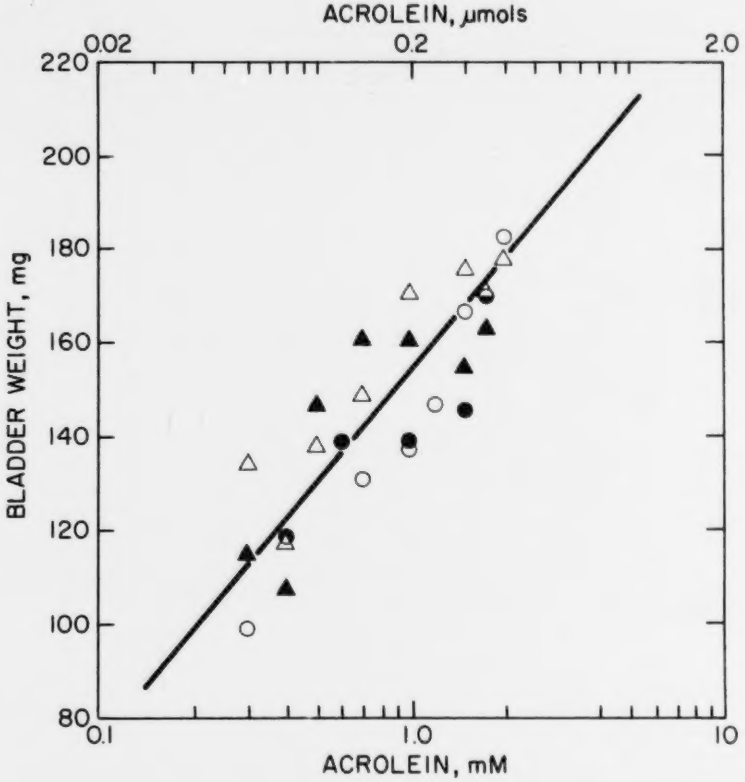

FIGURE 4.—Bladder toxicity effected by acrolein at pH 6 and 8 and at phosphate concentrations of 4 and 40 mM: dose-response relationship. Female Sprague-Dawley rats, 155–200 g, were anesthetized, their bladders were catheterized, and acrolein was infused in 0.2 ml of a 4 mM phosphate buffer solution at pH 6 (△) or 8 (▲) or a 40 mM phosphate buffer solution at pH 6 (○) or 8 (●). 1 hr later, infusates were removed, bladders were flushed with a normal saline solution, and catheters were removed. Bladder wet weights were determined 48 hrs after infusion. Acrolein was omitted from infusate to obtain control bladder weights. Values for control bladder weights are given in table 3. All values are the mean of determinations made on 2–6 animals. SE bars were omitted for purposes of clarity; they ranged from 4 to 22 mg.

significant ($P < 0.1$) increase in bladder weight when the pH was 6 and the phosphate concentration was 40 mM (table 3). Brock et al (1,3) reported similar findings.

Wagner et al (14) argue that acrolein liberated from 4-hydroxycyclophosphamide in the urine cannot be the causative agent with regard to cyclophosphamide-induced hemorrhagic cystitis, because the amounts of 4-hydroxycyclophosphamide and acrolein that they found in the urine were insufficient to account for the expected cystitis; no mention was made as to whether cystitis did indeed occur.

Urinary excretion of 4-hydroxycyclophosphamide following the administration of 50 mg/kg of cyclophosphamide ip to male rats is presented in figures 6 and 7. The assay employed is relatively specific for acrolein and compounds that give rise to acrolein, eg, 4-hydroxycyclophosphamide (7,8). We assume that the material determined to be present in the urine is largely or exclusively 4-hydroxycyclophosphamide because (*a*) acrolein generated in the plasma is likely to react immediately with plasma macromolecules and thus is not likely to be cleared as such from the plasma by the kidneys (12,15) and (*b*) the time between urine formation and collection is relatively short, so that little conversion of 4-hydroxycyclophosphamide to acrolein in the urine itself would be expected.

The urinary concentration of 4-hydroxycyclophosphamide in successive 30-min collection periods decreased exponentially and reflected the exponential decrease in plasma concentrations of 4-hydroxycyclophosphamide; the highest concentration observed was about 2 mM (fig 6). Assuming that the bladder is completely empty at the start of the experiment, that the animal does not void during the experiment, and that

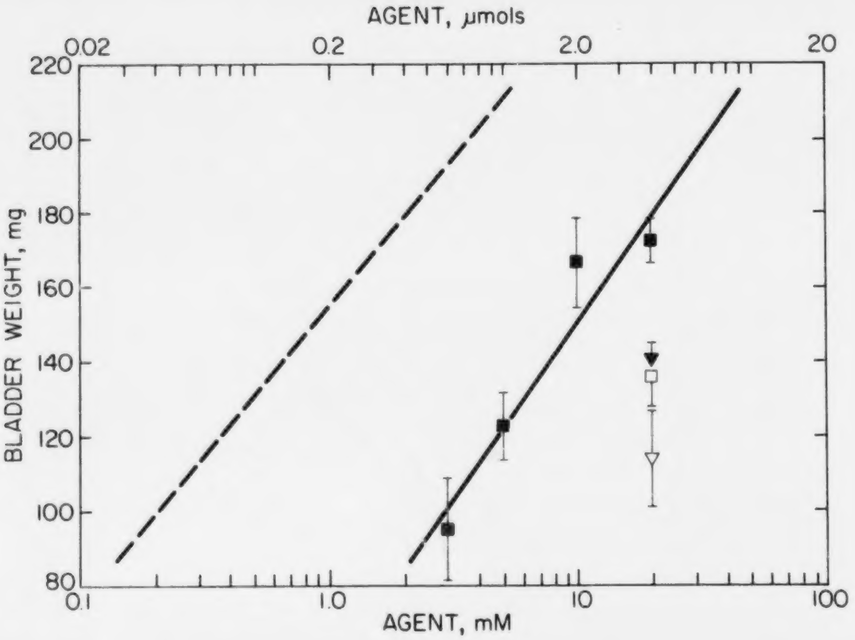

FIGURE 5.—Bladder toxicity effected by 4-hydroperoxycyclophosphamide at pH 6 and 8 and at phosphate concentrations of 4 and 40 mM. Female Sprague-Dawley rats, 155–200 g, were anesthetized, their bladders were catheterized, and 4-hydroperoxycyclophosphamide was infused in 0.2 ml of a 4 mM phosphate buffer solution at pH 6 (▽) or 8 (▼) or a 40 mM phosphate buffer solution at pH 6 (☐) or 8 (■). 1 hr later, infusates were removed, bladders were flushed with a normal saline solution, and catheters were removed. Bladder wet weights were determined 48 hrs after infusion. 4-Hydroperoxycyclophosphamide was omitted from the infusate to obtain control bladder weights (values listed in table 3). All values are the mean ± SE of determinations made on 3–6 animals. The dose-response relationship between acrolein and bladder weight (broken line) is included for comparison.

4-hydroxycyclophosphamide in the urine does not decompose, the cumulative urinary concentration of 4-hydroxycyclophosphamide in the bladder is calculated to range between 1 and 2 mM during the 3-hr period following cyclophosphamide administration (fig 6, inset). About 3.5% (2 µmols) of the total cyclophosphamide dose was excreted in the urine as 4-hydroxycyclophosphamide; most of this was excreted in the 3-hr period following cyclophosphamide administration (fig 7).

The values obtained in the present experiments for urinary 4-hydroxycyclophosphamide are somewhat higher than those obtained by others (14,16–18). Probable explanations relate to the dose of cyclophospha-

TABLE 3.—Bladder toxicity of phosphoramide mustard*

pH	PO_4 (mM)	Bladder weight (mg)	
		Control	Phosphoramide mustard
6	4	119 ± 10	117 ± 8
6	40	108 ± 8	138 ± 17†
8	4	111 ± 4	115 ± 15
8	40	104 ± 11	123 ± 21

*Female Sprague-Dawley rats, 155–200 g, were anesthetized, their bladders were catheterized, and 50 mM phosphoramide mustard (10 µmols) was infused in 0.2 ml of a 4 or 40 mM phosphate buffer solution at pH 6 or 8. 1 hr later, infusates were removed, bladders were flushed with normal saline solution, and catheters were removed. Bladder wet weights were determined 48 hrs after infusion. Phosphoramide mustard was omitted from infusate to obtain control bladder weights. Bladder weight in animals not submitted to infusion was 69 ± 4 mg. All values are the mean ± SE of determinations made on 3–4 animals.

†Significantly different from control; $P < 0.1$.

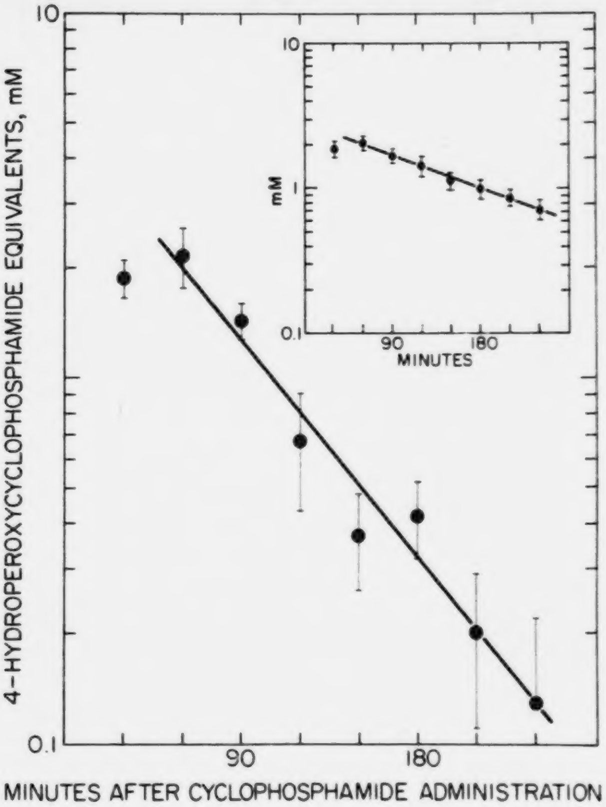

FIGURE 6.—Urinary 4-hydroxycyclophosphamide concentrations. Male Sprague-Dawley rats, 300–350 g, were prepared and injected with 50 mg/kg of cyclophosphamide as described in Materials and Methods section. Urine was collected for successive 30-min periods, and 4-hydroxycyclophosphamide concentration in each sample was determined as described in Materials and Methods section. Values are the mean ± SE of determinations made on 4 animals. Inset: Concentrations of 4-hydroxycyclophosphamide calculated to be present in bladder urine, assuming that bladder is empty at start of experiment and that animals do not void during 4-hr collection period.

mide employed, the relative rates at which 4-hydroxycyclophosphamide is generated and excreted in different species, and the stability of 4-hydroxycyclophosphamide in urine.

Administration of 50 mg/kg of cyclophosphamide ip to male and female rats increases bladder weight by about 30–40 mg/100 g of body weight (fig 1). In situ, approximately 0.26–0.42 μmol of acrolein is required to produce a similar response (fig 4). About 3.5% of the cyclophosphamide given to 325-g rats was excreted in the urine as 4-hydroxycyclophosphamide, the potential source of acrolein (fig 7). Assuming that 3.5% of the administered dose is also excreted in smaller rats, 1.0–1.7 μmols of 4-hydroxycyclophosphamide would be excreted in the urine of 150–250-g rats given 50 mg/kg of cyclophosphamide. The pH of the urine obtained from cyclophosphamide-treated male rats averaged about 6.8; the urinary inorganic phosphate and bicarbonate concentrations averaged about 32 mM and 3 mEq/L, respectively (table 1). Bicarbonate is essentially without catalytic activity at this concentration and pH (N. E. Sladek, unpublished observations), but the half-life of 4-hydroxycyclophosphamide in 32 mM phosphate at pH 6.8 is about 3 hrs (5). Thus, the amount of 4-hydroxycyclophosphamide (acrolein) excreted in the urine is clearly sufficient to account for cyclophosphamide-induced bladder toxicity.

DISCUSSION

Our investigations strongly support the view that the urotoxic effects of cyclophosphamide are caused by the acrolein liberated from 4-hydroxycyclophosphamide in the urine (1–3). The results clearly refute the contention

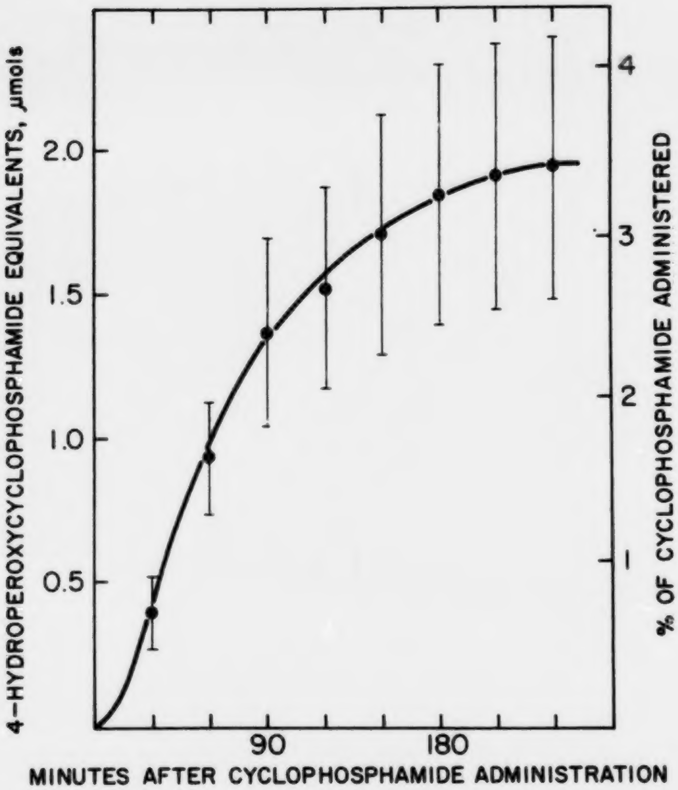

FIGURE 7.—Urinary excretion of 4-hydroxycyclophosphamide. Cumulative amount of 4-hydroxycyclophosphamide excreted in urine was determined as part of experiments presented in fig 6. Details of experiment are given in fig 6 and Materials and Methods section.

that the amounts of 4-hydroxycyclophosphamide and acrolein in the urine are insufficient to account for the toxicity (14). In all probability, only the acrolein released in the urine causes the toxic action because acrolein is very reactive and, when generated systemically, reaches the urine largely in the form of 3-hydroxypropylmercapturic acid, a relatively innocuous metabolite (12,15).

Conversion of 4-hydroxycyclophosphamide to acrolein requires the presence of a general base catalyst (4,5). Several, perhaps numerous, agents can serve in this capacity; identified general base catalysts include bicarbonate and inorganic phosphate (5). The rate at which the conversion to acrolein proceeds is highly dependent on the concentrations of these catalysts in the environment as well as on the pH of that environment; the rate increases as these environmental variables increase [(4,5); J. E. Low and N. E. Sladek, unpublished observations]. These considerations gave rise to the prediction that the urotoxic potency of 4-hydroxycyclophosphamide, and thus cyclophosphamide, would increase with increasing urinary pH and concentrations of general base catalysts (5). The experiments presented in figure 5 clearly demonstrate that this was the case.

Increased fluid intake, diuretics, alkalinization of the urine, perfusion of the bladder with nucleophiles, and systemic administration of rapidly excreted nucleophiles have all been offered as potentially useful preventive measures with regard to the urotoxic effects of cyclophosphamide (1,2,19–30). Our findings clearly indicate that alkalinizing the urine is contraindicated as is the use of certain diuretics. Specifically, whereas the use of diuretics such as NH_4Cl that do not alkalinize the urine or increase urinary general base catalyst concentration is likely to be beneficial in minimizing cyclophosphamide-induced bladder toxicity, the use of diuretics such as acetazolamide that do alkalinize the urine and/or increase urinary general base catalyst concentration may not be helpful, even though urine flow is increased. In the case of acetazolamide, a reduction in cyclophosphamide-induced urotoxicity would be expected, due to dilution of the reactants and decreased contact time caused by the increased urine flow. This is offset, however, by the marked increase in urinary pH and bicarbonate concentration; at 100 mEq/L of bicarbonate, the half-life of 4-hydroxycyclophosphamide is about 2 hrs at pH 8 (J. E. Low and N. E. Sladek, unpublished observation), and although the inorganic phosphate concentration decreases, its catalytic activity does not change appreciably, due to the increased pH (4,5).

The reader should be aware that the dose of furosemide used in our experiments was relatively high. In man, the acute effects of a therapeutic dose of furosemide do not usually include acidification of the urine and may even include a slight increase in urine pH; increased bicarbonate excretion and phosphaturia may also be observed (31).

From the above considerations, it follows that in addition to alkalinizing agents and certain diuretics, other factors may potentially influence the urotoxic potential of cyclophosphamide by increasing urinary bicarbonate or inorganic phosphate concentrations and/or by alkalinizing the urine. Included factors are other drugs, hormonal status, disease status, diet, age, and chronorhythmic variability. For example, other agents that inhibit carbonic anhydrase would increase bicarbonate excretion and alkalinize the urine, and insulin and parathyroid hormone are known to be antiphosphaturic and phosphaturic, respectively. The renal handling of phosphate and the phosphaturic potential of agents such as calcitonin, glucagon, dopamine, and acetylcholine, as well as those of other agents, have been reviewed (32).

General base catalysts other than phosphate and bicarbonate may also be operative (5). Disease and hormonal status, diet, age, chronorhythmic variability, and drugs would also be expected to influence the concentration of these catalysts in the urine, but specific possibilities cannot be identified because the identity of the putative catalysts is not currently known.

Ifosfamide, a chemical congener of cyclophosphamide, is also urotoxic; hemorrhagic cystitis is the major dose-limiting toxic reaction to this agent. This toxicity is caused by the acrolein released in the urine from 4-hydroxyifosfamide, the primary metabolite of ifosfamide (1,3). Since this conversion also requires the presence of a general base catalyst and is identically pH-dependent (J. E. Low and N. E. Sladek, unpublished observations), the considerations made above for cyclophosphamide are likely to apply to ifosfamide as well.

Cyclophosphamide is also, apparently, a bladder carcinogen (33–37). Whether acrolein generated in the urine is the causative agent is unclear. We are unaware of any studies directed at evaluating the carcinogenic potential of acrolein. However, as pointed out by Izard and Libermann (38), acrolein can give rise to glycidal via epoxidation (39), and the carcinogenicity of glycidal has been established (40). Alternatively, acrolein may sensitize the bladder epithelium to the putative carcinogenic action of one or more of the other urinary metabolites of cyclophosphamide that are, or can give rise to, alkylating agents; increased sensitization could be the result of damage to the bladder epithelium and the subsequent rapid increase in cell division caused by acrolein. In either case, the concentration of general base catalysts in, and the pH of, the urine would be important determinants relative to the potential of cyclophosphamide and other oxazaphosphorines to induce bladder cancers.

The systemic effects of cyclophosphamide are apparently mediated by phosphoramide mustard generated from 4-hydroxycyclophosphamide within cells, at least in those cells that are substantially more sensitive to 4-hydroxycyclophosphamide than to phosphoramide mustard when these agents are added directly to the environment (41–43). A similar argument can be made for other oxazaphosphorines. Cyclophosphamide has, among antitumor and immunosuppressive agents, a relatively good therapeutic index. The basis for this desired property has not been established but apparently resides with 4-hydroxycyclophosphamide (41,42). Several possibilities have been advanced and reviewed (42,44). We have recently suggested yet another possibility (5). The other metabolite released when 4-hydroxycyclophosphamide gives rise to acrolein is phosphoramide mustard; some cells may be differentially sensitive to 4-hydroxycyclophosphamide because of a greater ability by these cells to convert 4-hydroxycyclophosphamide to phosphoramide mustard, this ability stemming from a relatively greater intracellular concentration of general base catalysts and/or pH. Our investigations in the bladder could be viewed as experiments in a model system that give credence to this possibility.

The potential importance of general base catalysts and pH in the urine and in the extracellular and intracellular spaces with regard to the localized release of toxic (carcinogenic) metabolites from relatively inert parent compounds should also be noted. These factors may account for the species, strain, organ, or cellular specificity of some agents.

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Editorial on Management of Drug Toxicity

The remarkable advances of the past 20 years in the drug treatment of malignant disease have been purchased at a price, namely the short- and long-term toxic effects of the drugs that are employed. The therapeutic index of many agents is extremely narrow and there is significant overlap between tumor cytotoxicity and toxicity for normal tissue. Emphasis in drug development has been placed upon the identification of chemicals that are actively cytocidal to cancer cells, and management of systemic toxicity by methods other than manipulation of drug dose and schedule frequently lags well behind the exploration of antitumor activity. In part, this is because modification of toxicity may require a detailed and sophisticated knowledge of drug pharmacology and a clear appreciation of whether the mechanisms of tumor cell kill and organ toxicity can be separately manipulated. Such studies are not likely to be undertaken until a drug has proved its worth in the clinic. However, as a drug or class of agents becomes well-established, interest rises in ameliorating its specific toxicity in order to improve the therapeutic index. Alkylating agents, anthracyclines, platinum analogs, and antimetabolites all have characteristic organ toxicities, and in each case research programs exist with the aim of understanding and eliminating these side effects. For the hemorrhagic cystitis produced by cyclophosphamide and its congener ifosfamide, work over the past decade has emphasized the role of sulfhydryl compounds such as *N*-acetylcysteine as well as the induction of a liberal diuresis to prevent this side effect. While studies in animals and man (1–3) indicate that systemic or intravesical instillation of sulfhydryl compounds may reduce cyclophosphamide-induced cystitis, these have never been widely used procedures, in part because systemic use of sulfhydryl compounds may also reduce the antitumor activity of the drugs (4). Another standard recommendation is to induce diuresis, either with parenteral or oral fluids, to assist the excretion of cyclophosphamide metabolites and reduce their concentration in the urine. In the preceding article, Sladek and colleagues (5) suggest that acidification of the urine is desirable as well. Their studies in rats indicate that release of urotoxic acrolein is increased in the presence of general base catalysts such as phosphate and bicarbonate and that this reaction proceeds more effectively at an alkaline pH. Diuretics that acidify the urine minimize or eliminate the urotoxicity of cyclophosphamide, while acetazolamide, which alkalinizes the urine, does not alter toxicity. Systemic action of the drug is not altered in either case. These findings deserve study in man, as they may have immediate significance regarding the use of cyclophosphamide, especially in high doses. This may be a point at which the mechanisms of toxicity and antitumor activity of cyclophosphamide and its congeners diverge and can be independently managed.

Such findings emphasize the need for continued alertness by clinical investigators to the nuances of drug pharmacology. Other drugs, notably the platinum compounds and methotrexate, are also urotoxic. Recently Ozols et al (6) noted that the renal toxicity of high cisplatin doses could be ameliorated by the concurrent administration of hypertonic saline. If this finding is confirmed it will be another useful addition to our knowledge in the use of platinum compounds. On the other hand, it is known that methotrexate crystallizes in renal tubules at acid pH and that alkalinization of the urine is an important part of the prevention of renal damage by high-dose methotrexate (7).

Other investigators are currently examining the mechanisms and means of eliminating important drug toxicity such as the cardiac toxicity of anthracyclines, the severe cutaneous damage of anthracyclines and actinomycin, and the myelotoxicity of antimetabolites and alkylating agents. Since the recognition of novel new drugs for treatment of cancer is often a slow process, some of the important gains in the near future may come from learning to diminish toxicity and thereby widen the therapeutic index of the anticancer drugs presently available.

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Paul V. Woolley

Georgetown University Medical Center
Vincent T. Lombardi Cancer Research Center
Washington, DC 20007

September 14, 1982

Intracavitary Bleomycin in the Management of Malignant Effusions: A Multicenter Study[1]

M. J. Ostrowski[2,*] and G. M. Halsall[3]

The efficacy of intracavitary bleomycin in preventing the recurrence of malignant effusions following aspiration was assessed in a multicenter study. Of 200 patients treated, 158 were evaluated for response at 30 days. The overall response rate was 58%, with pleural effusions responding better (62%) than peritoneal effusions (47%). Pleural effusions resulting from primary breast tumors showed the best response (72%). No side effects were seen in 79.5% of the 200 patients. Pain and transient fever were reported in 21% of the patients after intraperitoneal instillation but in only 5% of those receiving intrapleural instillations. Nausea was experienced by 5.5% of the patients. There was no evidence of myelosuppression in any patient nor of enhancement of myelosuppression in the 57 patients receiving concurrent cytotoxic therapy. There was one possible treatment-related death in an elderly man given 120 mg of bleomycin intrapleurally. This leads us to recommend that the maximum dose should be 60 mg, especially as the response rate was not improved by doses > 60 mg. We conclude that bleomycin should be the agent of choice when the instillation of a cytotoxic agent following the drainage of a malignant effusion is indicated, since it is effective in preventing recurrence of the effusion, is generally free from systemic effects, and can be given to myelosuppressed patients or those already undergoing systemic cytotoxic therapy. [Cancer Treat Rep 66:1903–1907, 1982]

Serous effusions result when the dynamic balance between fluid formation and resorption within the serous space is disrupted. The frequency with which effusions occur as a complication of progressive neoplastic disease has not been determined, but Leff et al (1) stated that 25% of all effusions are due to neoplasia. Relief of dyspnea, cough, and pain usually follows aspiration of the effusion, but reaccumulation of fluid is rapid. In one study (2) of 94 patients treated by thoracentesis alone, the average time for reaccumulation of the effusion was 4.2 days, with a 97% chance of recurrence within 1 month. Repeated aspirations may induce hypoproteinemia, which can accelerate the reaccumulation of fluid.

Different techniques have been used to prevent or delay the recurrence of malignant serous effusions, including closed-tube thoracostomy with supplementary suction, external radiotherapy, or instillation of one of a variety of agents such as radioactive isotopes and sclerosing and cytotoxic drugs. Response rates of 50%–70% have been obtained using these forms of treatment (2), but there are problems associated with their use: tube thoracostomy is a time-consuming technique; radioactive isotopes subject both hospital personnel and fellow patients to the risk of radioactive exposure; cytotoxic agents may give rise to bone marrow suppression, nausea, and vomiting; and sclerosing agents tend to be poorly tolerated because of pain.

Because of these disadvantages there is a need for an agent that is effective, easy to administer, and free from side effects. Bleomycin is a cytotoxic agent that has been shown to be effective in a number of small studies (3,4–8), and so a multicenter study was begun to investigate further the role of intracavitary bleomycin in a larger number of patients with malignant serous effusions secondary to a variety of tumors and to determine the optimum dose for instillation.

PATIENTS AND METHODS

Two hundred patients with histologically documented malignancy and pleural and/or peritoneal effusions were entered into this study from 44 centers in the United

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[1] Received July 31, 1981; revised June 24, 1982; accepted Aug 5, 1982.

[2] Norfolk and Norwich Hospital, Norwich, England.

[3] Lundbeck Limited, Luton, Bedfordshire, England.

[*] *Reprint requests to:* Dr. M. J. Ostrowski, Department of Radiotherapy and Oncology, Norfolk and Norwich Hospital, Brunswick Rd, Norwich, Norfolk, NR1 3SR, England.

Kingdom, each clinician treating between three and five patients. The aim of the study was to evaluate the use of simple pleural aspiration and peritoneal drainage followed by intracavitary bleomycin in busy general hospitals, as well as in the more academic units. The information recorded on entry to the study included age and sex of the patient, duration of the effusion, site and histology of the primary tumor, cytologic examination of the effusion, details of concurrent treatment, and status of the patient's primary tumor.

Aspiration Techniques

Pleural effusions.—Drainage was as complete as possible, as confirmed by chest X-ray, with appropriate precautions taken when the volume exceeded $1^1/_2$ L. Drainage was followed by the instillation of bleomycin in 100 ml of physiologic saline. The most common technique used was cannula or needle aspiration over 30-60 minutes.

Peritoneal effusions.—Gravity drainage to dryness over 12-24 hours was followed by the instillation of bleomycin in 100 ml of physiologic saline.

Dose.—Inadequate data were available regarding the most effective dose to use. Clinicians were asked to select, at their own discretion, a dose within the range of 30-180 mg.

There is no standard assessment of response recognized in the literature; in this survey the criteria used by Paladine et al (4) were adopted so that our results would be comparable. A complete response was defined as no further reaccumulation of fluid within 30 days, and a partial response was defined as asymptomatic, minimal fluid recurrence not requiring a repeat aspiration within 30 days. Failure was defined as reaccumulation of the fluid within 30 days, requiring further aspiration. Recurrences of effusion were determined by regular clinical assessment and chest X-rays. A full assessment of response to bleomycin was made at 1 month (30 days) but long-term results were not routinely recorded in view of the variety of primary tumor types treated, the different stages of the disease on entry, and the limited life expectancy of many patients. However, some clinicians were able to provide follow-up information at 60 and 90 days after instillation of bleomycin.

Of the 200 patients with malignant effusions entered into this study, 42 were excluded from analysis for the following reasons: ten died of progressive disease < 30 days after treatment with bleomycin and before response could be assessed, 26 died of progressive disease in < 30 days but without recurrence of their effusion, and six, all without recurrence, were reviewed prematurely before the 30-day assessment period (range, 17-29 days). This left 158 evaluable patients, seven of whom were lost to follow-up and have been included as nonresponders for the purpose of this analysis. Of these 158 patients, there were 66 with primary breast tumors, 30 with ovarian lesions, 27 with lung cancer, and 35 with miscellaneous tumors.

RESULTS

The overall response rate at 30 days was 58% (91 patients), 35% complete responders and 23% partial. Pleural effusions responded better (62%) than peritoneal effusions (47%) (table 1). This may be because in advanced disease, peritoneal deposits invariably become large and confluent so that the opportunity for uniform contact between drug and tumor may be less favorable in the peritoneal cavity than in the pleural space. Another reason might be obstruction of hepatic lymphatics, which would not be expected to respond to intraperitoneal therapy.

Pleural effusions associated with breast primaries were the most responsive to intracavitary bleomycin, with a response rate of 72%. Only 37% of the patients with pleural effusions secondary to lung tumors responded,

TABLE 1.—Response rate by site of primary tumor and serous cavity affected ($n = 158$)

	Site of primary tumor												Response rate by site of effusion		
	Breast			Ovary			Lung			Miscellaneous					
Site of effusion	CR	PR	CR + PR	CR	PR	CR + PR	CR	PR	CR + PR	CR	PR	CR + PR	CR	PR	CR + PR
Pleural	46%	26%	72% ($n = 54$)	—	2 of 3	2 of 3	22%	15%	37% ($n = 27$)	36%	32%	68% ($n = 22$)	37%	25%	62% ($n = 106$)
Peritoneal	45.5%	18.5%	64% ($n = 11$)	22%	17%	39% ($n = 23$)		—		31%	15%	46% ($n = 13$)	30%	17%	47% ($n = 47$)
Pleural and peritoneal	1 of 1		1 of 1	1 of 4	1 of 4	2 of 4		—			—		2 of 5	1 of 5	3 of 5
Response rate by site of primary tumor	47%	24%	71% ($n = 66$)	20%	23%	43% ($n = 30$)	22%	15%	37% ($n = 27$)	34%	26%	60% ($n = 35$)			

but this included eight of ten patients with squamous cell tumors, which, in view of the well-known affinity of bleomycin for squamous cells, may indicate a specific local effect. Of the pleural effusions due to miscellaneous tumors, 68% responded although the sensitivity of individual tumor types cannot be determined from such a small series (table 2).

There were 47 patients with ascites available for evaluation, of whom 22 (47%) responded to treatment with bleomycin. As in the case of pleural effusions, patients with primary carcinoma of the breast responded well, with a response rate of 64% (seven of 11). Five patients (one with a breast primary and four with ovarian primaries) had both pleural and peritoneal effusions. Three of these patients responded to treatment, with relief of both pleural and peritoneal effusions.

The duration of response cannot be fully assessed in this preliminary evaluation. Of the 91 responders at 30 days, 66 patients were symptom-free at 60 days, five had died, six had been retapped, and 14 were lost to follow-up. Of the 66 symptom-free patients, on whom complete follow-up information was available at 60 days, 32 were still alive and well with no further effusion at 90 days, seven had died, six had been retapped, and 21 were lost to follow-up.

Toxicity

No side effects were reported in 159 (79.5%) of the 200 patients entered into this study. Pain and fever were the most commonly reported side effects, occurring in 10.5% of the patients. In most cases, these two symptoms occurred together. A total of 21% of the patients receiving intraperitoneal bleomycin experienced pain, in contrast to only 5% of those given the drug intrapleurally. Pain was controlled by mild analgesics in seven cases but was severe in another seven. (The severity of pain in the remaining seven cases was not recorded.) The causes of pain may have included irritation by bleomycin, producing an inflammatory response; the cannulation procedure; or injection of bleomycin into the abdominal wall. Gastrointestinal side effects were experienced in 5.5% of the patients (diarrhea in one, vomiting in two, and nausea in eight), whereas erythema and dyspnea, which have occasionally been seen with the parenteral administration of bleomycin, were each reported in only one patient. Two of the three patients who developed alopecia were receiving concurrent chemotherapy with cyclophosphamide. There was one death, possibly related to treatment, in a 75-year-old man given 120 mg of bleomycin intrapleurally. He became ill on the following day, with labored respiration, a rapid pulse, and a blood pressure of 116/60. The ECG was suggestive of a myocardial infarction, and he died 3 days later.

Dose

The dose of bleomycin varied between 30 and 180 mg, with 60, 90, and 120 mg being most frequently used. Of 81 patients receiving a dose of 60 mg, 52 showed a response and 29 did not. Fifteen of 29 patients who were given a dose of 90 mg responded, and 120 mg produced a response in 11 of 19 patients. There was no difference in the mean dose of bleomycin given to responders (mean, 72 mg; range, 30–180) and nonresponders (mean, 74 mg; range, 30–150). The median dose was 60 mg in both responders and nonresponders, which suggests that 60 mg is a reasonable routine dose to administer.

TABLE 2.—Sites of miscellaneous primary tumors and response of effusion to bleomycin

	Pleural effusions (n = 22)		Peritoneal effusions (n = 13)	
	Responders	Nonresponders	Responders	Nonresponders
Colorectal adenocarcinoma	2	—	1	2
Esophageal adenocarcinoma	—	—	—	1
Gastric adenocarcinoma	—	—	—	1*
Non-Hodgkin's lymphoma	3	—	—	—
Hodgkin's disease	1	—	—	—
Melanoma	1	—	—	—
Adamantinoma	1	—	—	—
Osteosarcoma	—	1	—	—
Mesothelioma	3	3	—	—
Endometrial carcinoma	1	1	3	—
Unknown primary	3	2*	2	3

*1 patient lost to follow-up and included as a nonresponder.

The development of a malignant effusion indicates progression of disease, even though in some cases the patient is in good general health and may have many months of good-quality life remaining. In other patients, however, it signifies the entry into the terminal phase of their illness, so their life expectancy is limited. In this series of patients, 76 had uncontrolled advancing disease (ie, the development of an effusion was only one of several signs of progressive disease), in 57 the other signs of disease were present but static, and in 17 the effusion was the only sign of progressive disease. Table 3 shows the response of the effusion to intracavitary bleomycin in relation to the other signs of the patient's disease. The patients in whom an effusion was the only sign of advancing disease responded well to bleomycin therapy (16 of 17 patients). The effusion was controlled in 39 of the 76 patients with uncontrolled progressive disease and in 36 of the 57 patients in whom the other signs of disease were static. Whether or not other signs of disease are progressing or in remission appears therefore to be a prognostic factor of importance for prediction of response to bleomycin ($P < 0.01$).

Many patients were receiving concurrent systemic chemotherapy for their disease, and it could be argued that this treatment may have influenced the effusion-free interval. However, systemic chemotherapy was not changed prior to or during the trial, so that in some patients the effusion was accumulating despite concurrent chemotherapy. In fact, there were only small differences between the concurrent chemotherapy given to those patients whose effusion was controlled by bleomycin (bleomycin responders) and those patients whose effusion was not controlled by bleomycin (bleomycin nonresponders). For instance, 13% of bleomycin responders were given corticosteroid, 9% were given diuretics, and 1% received estrogens. Among bleomycin nonresponders, 18% were given corticosteroid, 17% received diuretics, and 5% had radiotherapy.

Concurrent cytotoxic chemotherapy was administered to 57 patients in whom there was no evidence of enhanced myelosuppression. The percentages of patients who received cytotoxic chemotherapy and whose effusion was controlled or not by intracavitary bleomycin are similar (37% and 38%, respectively). Twenty-one patients, all with carcinoma of the breast, were taking the antiestrogen tamoxifen, and the control of effusion by bleomycin in this group was very good (81%). This response rate is comparable to that seen in the whole group of patients with breast cancer (71%). Of the bleomycin responders, 37% were actually receiving no concurrent chemotherapy, compared with only 28% of the bleomycin nonresponders. These figures indicate that the control of effusion in this series of patients was due not to systemic chemotherapy but to intracavitary bleomycin.

TABLE 3.—Status of other signs of malignancy*

Status of other signs of malignancy	Effusion responding to intracavitary bleomycin (n = 91)	Effusion not responding to intracavitary bleomycin (n = 60)†
Progression	39 (43%)	37 (62%)
No change	36 (39.5%)	21 (35%)
Remission	16 (17.5%)	1 (1.5%)

*$\chi^2 = 10.927$; df = 2; $P < 0.01$.
†Information not available for 1 patient.

The mean and median duration of effusion before treatment with bleomycin was the same in responders (mean, 4 months; median, 1; range, 0.5–36) and nonresponders (mean, 3.2 months; median, 2; range, 0.5–25). Age was not a determining factor in predicting response, the mean age being 60 years (range, 33–82) in responders and 61 years (range, 17–84) in nonresponders.

Cytologic assessment of the effusion was not done in 35% of the patients (33% of the responders and 37% of the nonresponders) and may not, therefore, be representative of the whole group of patients. However, of the responders, 77% had malignant cells in the aspirated fluid and 23% did not. Of the nonresponders, 74% were cytologically positive and 26% negative. Bleomycin appears therefore to be equally effective both in the presence and absence of malignant cells in the effusion.

DISCUSSION

Cunningham et al (3) first reported the use of intracavitary bleomycin in the management of malignant effusions in 12 patients, of whom five (48%) showed a complete response. Patient tolerance was described as excellent; only one person reported a transient mild erythematous rash, and there was no exacerbation of leukopenia, which was present in two patients prior to treatment. Paladine et al (4) confirmed this initial impression in their series of 51 patients who had an overall response rate of 59% at 30 days. Pleural effusions responded better than peritoneal effusions, with response rates of 63% and 36%, respectively; 70% of the pleural effusions secondary to primary breast carcinoma responded. Toxicity was described as minimal. Alopecia, skin changes, and pulmonary fibrosis were not seen despite evidence of systemic absorption; pain, nausea, rashes, or hypotension occurred in only 2% of the patients; and ten patients (20%) experienced a transient fever. Toxicity was more common following intraperitoneal instillation and was thought to be due to the greater drug absorption from the peritoneal cavity (5,9). The absence of hematologic toxicity and local discomfort led the authors to conclude that bleomycin is an effective alternative to quinacrine for the control of pleural effusions in the leukopenic patient.

Three abstracts also reported the use of intracavitary bleomycin, all in small series of patients. Lippman et al (6) claimed a 92% response rate (11 of 12 patients) in pleural effusions but three of three peritoneal effusions failed to respond. There were no toxic reactions. Trotter et al (7) reported to the British Association of Cancer Research a study of 22 patients who received intracavitary bleomycin for control of malignant effusions: 78% of pleural effusions and 69% of peritoneal effusions responded. There were two possible drug-related deaths, one of which was associated with an unusually high serum level of bleomycin. This led them to recommend that the dose of bleomycin not exceed 40 mg/m^2 in the elderly. In our series there was also one possible drug-related death in a patient given 120 mg of bleomycin intrapleurally, and we endorse this recommendation, particularly as the response rate is not improved by doses > 60 mg. A study by Gupta et al (8) comparing intrapleural bleomycin in 13 patients and intrapleural tetracycline in 12 patients showed response rates of 54% (seven of 13) and 58% (seven of 12), respectively. They concluded that the agents are equally effective with minimal toxicity.

The mechanism of action of intracavitary bleomycin is unknown. Systemic absorption of intracavitary bleomycin is approximately 45% of the instilled dose (9). The half-life after intraperitoneal instillation is significantly longer than after intrapleural instillation, which in turn is significantly longer than after iv injection (9). The longer half-life following intraperitoneal instillation as compared with intrapleural instillation may be due to incomplete drainage of peritoneal fluid prior to instillation of bleomycin. Dedrick et al (10) suggest that the systemic absorption of a drug from the peritoneal cavity is dependent on the volume of peritoneal fluid: the larger the residual peritoneal fluid volume, the lower the rate of drug absorption.

Bleomycin may therefore remain in contact with the abdominal serous membrane for a relatively long time, and this might explain the more frequent observation of pain after intraperitoneal instillation (21%) than after intrapleural instillation (5%) in this study. However, pleural effusions respond better than peritoneal effusions to intracavitary bleomycin, so this extended contact of the serous membranes with the drug does not appear to be necessary for bleomycin to exert its therapeutic effect. The fibrous adhesions found at autopsy suggest that bleomycin may stimulate an irritative pleuritis, although a local cytotoxic effect cannot be ruled out (4). An interesting observation in our study was that eight of ten patients with pleural effusions secondary to squamous cell lung primaries responded, suggesting that bleomycin may have a local cytotoxic effect.

The place of bleomycin in the management of malignant effusions can be determined only by controlled comparative clinical trials, using standard aspirating techniques and carefully defined response criteria. However, the results of this study in a large number of patients confirm that bleomycin is effective in the palliation of serous effusions arising secondary to a wide range of malignancies, and indicate that it may have advantages over other agents. The drug is well-tolerated, which is an important factor in treating patients with advanced malignant disease in whom myelosuppression, pain, nausea, and vomiting would detract from their quality of life. For these reasons, we conclude that bleomycin should be the agent of choice when the instillation of a cytotoxic agent following the aspiration of a malignant effusion is considered appropriate.

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Hydroxy-9-methyl-2-ellipticinium for Osseous Metastases From Breast Cancer: A 5-Year Experience[1]

P. Juret,* J. F. Heron, J. E. Couette, T. Delozier, and J. Y. Le Talaer[2]

A group of 135 patients with osseous metastases from breast cancer were treated with hydroxy-9-methyl-2-ellipticinium (100 mg/m^2 weekly). Although it was impossible to grade the response precisely, because only indirect criteria are available for assessing the course of bone metastases (radiographs, quantified ^{99m}Tc pyrophosphate scintigrams, CEA), it was considered that an objective response was obtained in 44 cases. These responses lasted from 3 to 17 months. The main characteristic of the compound is its lack of marrow toxicity, a valuable property in osseous lesions, where frequent marrow involvement makes it difficult to use conventional drugs. The major and most unpleasant side effect was an inhibition of salivary secretion, which causes other complications such as tongue mycosis, anorexia, and asthenia. Immunologic disorders were less frequent, and four patients developed severe tubular renal insufficiency. [Cancer Treat Rep 66:1909–1916, 1982]

The antitumor action of ellipticine and its derivatives in animals has been reported by several authors (1–4). In human oncology, methoxy-ellipticine has been used successfully in the treatment of acute myeloblastic leukemia (5,6). In previous papers, our group pointed out the therapeutic value of hydroxy-9-methyl-2-ellipticinium (hereafter referred to as elliptinium) in advanced cancers, especially in breast cancers (7–10) (fig 1). This compound was synthesized and studied in experimental tumors by the Laboratoire de Chimiothérapie Expérimentale, Institut Gustave Roussy (Villejuif, France) and the Institut de Chimie des Substances Naturelles, Centre National de Recherches Scientifiques (Gif-sur-Yvette, France) (2–4). It is currently being used in phase II and III trials in several countries. The phase II studies have demonstrated some activity of elliptinium in thyroid and kidney carcinomas, in soft tissue sarcomas, and mainly in advanced breast cancers (8,9,11,12). However, to our knowledge, these trials have not included an evaluation of the effect of the compound on osseous metastases. This exclusion is understandable, since it is difficult to assess and measure the effect of any treatment on metastatic bone lesions because investigators have recourse only to indirect criteria (X-ray films, ^{99m}Tc pyrophosphate scintigrams, and biologic markers such as CEA).

These methods preclude grading responses by the criteria typically used in phase II trials, ie, complete remission, partial remission (> 50% and < 50%), or no remission. Nonetheless, osseous metastases are the most frequent secondary lesions of breast cancer, and we previously drew attention to the favorable effect of el-

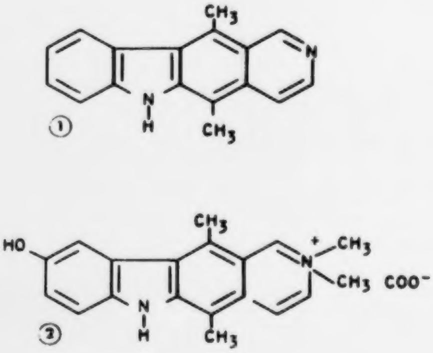

FIGURE 1.—Chemical structure of ellipticines: 1. ellipticine; and 2. hydroxy-9-methyl-2-ellipticinium acetate (elliptinium).

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[1] Received Oct 2, 1981; revised Aug 3, 1982; accepted Aug 19, 1982.

[2] Departments of Medicine (*P. Juret* and *J. F. Heron*), Nuclear Medicine (*J. E. Couette*), and Radiation (*T. Delozier*), and Biochemistry Laboratory (*J. Y. Le Talaer*), Centre François Baclesse, Caen, France.

**Reprint requests to:* Dr. Paul Juret, Medicine Department, Centre François Baclesse, Route de Lion-sur-Mer, F-14021 Caen Cedex, France.

liptinium in this sort of malignancy (7,8). This therapeutic value of elliptinium was enhanced by its low hematologic toxicity, a useful property in bone metastases, where the frequent involvement of the marrow often limits the use of conventional drugs. These considerations encouraged us to pursue the study of the effect of elliptinium in osseous metastases from breast cancer, in spite of the difficulty in assessing response.

MATERIALS AND METHODS

Between June 1976 and June 1981, 328 patients with advanced cancers were treated with elliptinium at the Centre François Baclesse (CFB). Among these patients, 183 had advanced breast carcinomas; in 137 of these, skeletal involvement was apparently the sole metastatic process as demonstrated by clinical examination, hepatic isotopic and/or ultrasonic scans, liver enzymes, and lung roentgenograms. Except for the first eight patients, who were initially included in phase I trial and received elliptinium at weekly doses of 4–80 mg/m^2, all patients were given a 60–90-minute weekly infusion of 100 mg/m^2 of elliptinium in an isotonic glucose solution. We chose the dose of 100 mg/m^2/week because our phase I study showed that this dose was usually well-tolerated (8). Two of the 137 patients were excluded from our study: one erroneously received an associated systemic treatment; the other was the only male in this series and his response will be considered separately. Therefore, a total of 135 women were included in this study. For eight patients, elliptinium had to be discontinued before the sixth week of treatment, because of intolerance (seven patients had digestive disorders, and one had hemolysis resulting from a rise of anti-elliptinium antibodies); they were considered failures. The remaining 127 women received a minimum of six weekly infusions, the treatment being continued until it became obvious that elliptinium was ineffective or that its effectiveness had ceased after a time of response. Treatment ranged from 6 to 86 weekly infusions.

With the exception of women known to have no estrogen receptors in their breast tumors, all patients underwent an endocrine treatment as a first systemic procedure (castration, tamoxifen). Chemotherapy was administered only after failure to respond to the endocrine treatment was ascertained or in the case of recurrent disease. From June 1976 to October 1978, patients were given elliptinium only after they had failed to respond to conventional chemotherapy (doxorubicin, cyclophosphamide, antimetabolites, etc) or when these cytostatic agents had to be discontinued due to their toxic effects (series 1). From October 1978, when it became clear that elliptinium could induce remission without marrow toxicity, the order of these last procedures was reversed: elliptinium became the first-line cytostatic treatment and conventional chemotherapy the second-line treatment (series 2). We excluded from this trial only patients whose poor vein system made cyclic infusions difficult. A total of 69 patients were treated in series 1 and 66 in series 2. No radiotherapy or other anticancer treatment was given jointly with elliptinium.

The main difficulty of this study was in the assessment of an objective remission, as the improvement or aggravation of osseous metastases can generally be established only by indirect methods. We based our evaluation on three different investigations:

(*a*) Monthly roentgenograms of the skull, whole spine, and pelvis (and other sites when necessary).

(*b*) Monthly ^{99m}Tc pyrophosphate scintigrams. The results were quantified according to the method elaborated at the CFB: the ratio of the uptake of this marker on healthy bones to the uptake on knees ranges normally from 1 to 2.5 but is usually higher in the case of metastatic lesion; aggravation or improvement results respectively in a rise or fall of the initial ratio (13).

(*c*) Twice monthly, CEA level was assayed (normal value: < 20 ng/ml).

The difficulty in evaluating an objective remission was increased by the fact that the above criteria were not constantly found. Of the 135 patients, 31 had normal scintigrams and 54 had normal CEA levels. Radiographic evidence of disease was present *in all patients*, but the X-ray images were difficult to compare in 25 cases, mainly when the lesions were situated in the sacropelvic area.

Given these conditions, response to elliptinium was rated as positive when the following occurred.

(*a*) Improvement according to *all the available criteria* and demonstrated by the above methods had lasted > 3 months: ie, an improvement of osteolytic or osteoblastic images on X-ray films *whatever the extent of that improvement*, a drop in an abnormal ^{99m}Tc pyrophosphate uptake ratio to the normal level, and a drop in CEA to < 20 ng/ml. In patients for whom two or three tests were available, a discrepancy of the results (eg, an improvement of CEA levels and scintigrams, but an aggravation of X-ray images) disqualified them from being considered as having positive responses. The three criteria were available for 58 patients, whereas only two criteria were available for 62 patients and only one criterion was available for 15 patients (table 1).

(*b*) No new metastatic lesion had appeared in any site (osseous, visceral, soft tissue, etc) during this period.

We are fully aware that this method of evaluation is subject to criticism, but the criticism is more theoretic than practical. In no case did we have any difficulty in classifying response as a remission or a failure.

RESULTS

Therapeutic Results

Therapeutic results are summarized in tables 1 and 2. Some examples of radiographic, scintigraphic, or biologic (CEA) remissions are given in figures 2–7.

In all cases, a partial or complete subjective remission was obtained with the objective response, with disappearance of pain and return to normal or subnormal activity. Response to treatment was demonstrated radiographically in 37 of 116 cases with evaluable images: in 34 of 103 cases with osteolytic images and in three of 13 cases with osteoblastic images. The other seven objective responses (images not evaluable on X-ray films) were demonstrated by the normalization of bone scans (two cases), CEA level (one), or both (four) (table 1). A relapse developing after a response was never ascertained on X-ray images but rather by a rise of CEA level, by an aggravation of scintigram, or by visceral or soft tissue metastases. No relationship could be found between response to elliptinium and previous response to hormonal treatment. The only male treated with elliptinium was excluded from the above series. He had an objective response which lasted 13 months.

As mentioned above, the present method of evaluation based on indirect criteria did not permit us to grade the responses. The only parameter that appeared to be

TABLE 1.—Objective responses to elliptinium in osseous metastases from breast cancer, in relation to the tests available for evaluation

Evaluation criteria	No. of patients		
	Total	Responding	With disease progression
1 test	15	4	11
X-ray films	6	1	
Scintigrams	5	2	
CEA	4	1	
2 tests	62	20	42
X-ray films + scintigram	37	9	
X-ray films + CEA	15	7	
Scintigram + CEA	10	4	
All 3 tests	58	20	38
X-ray films + scintigram + CEA			

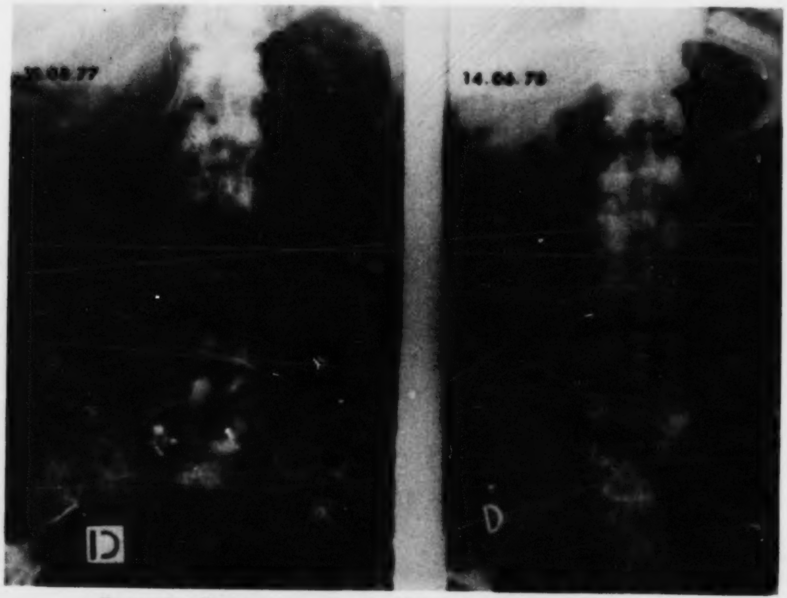

FIGURE 2.—Objective response after 10 mos of treatment with elliptinium.

measurable was the duration of the 44 apparent responses: > 6 months (39 patients) and > 12 months (five).

Toxic Effects

The side effects of elliptinium are summarized in table 3. We confirmed the findings reported in our previous papers as well as in papers published by other groups (7,8,11,12,14). These complications were the same in our series of osseous metastases and our overall series of 328 patients.

Hematologic toxicity.—The lack of marrow toxicity appears to be the most important characteristic of elliptinium. In fact, patients with leukopenia of < 1800 wbcs/mm^3 or thrombocytopenia of < 20,000 platelets/

TABLE 2.—Objective response to elliptinium in osseous metastases from breast cancer, in relation to the place of this compound in the therapeutic sequence[*]

Series	Therapeutic sequence	No. of patients	No. of responses (%)	Remission: Duration (mos)	Remission: No. of patients	Comments
1	Elliptinium as last treatment after hormonal procedures	69	19 (27)	3–6	2	In 4 patients, elliptinium was discontinued before the 6th infusion, because of intolerance; they were considered as failures
				6–9	6	
				9–12	9	
				> 12[†]	2	
2	Elliptinium as 1st systemic treatment after hormonal procedures	66	25 (37)	3–6	3	In 3 patients, elliptinium was discontinued before the 6th infusion, because of intolerance; they were considered as failures In 4 of the 25 patients, when response ceased, further remission was obtained with conventional chemotherapy
				6–9	7	
				9–12	12	
				> 12[‡]	3	
1 and 2		135	44 (32)			

[*] All patients were previously treated with hormonal procedures except when estrogen receptors were known to be absent.

[†] 12–15 mos.

[‡] 12–17 mos.

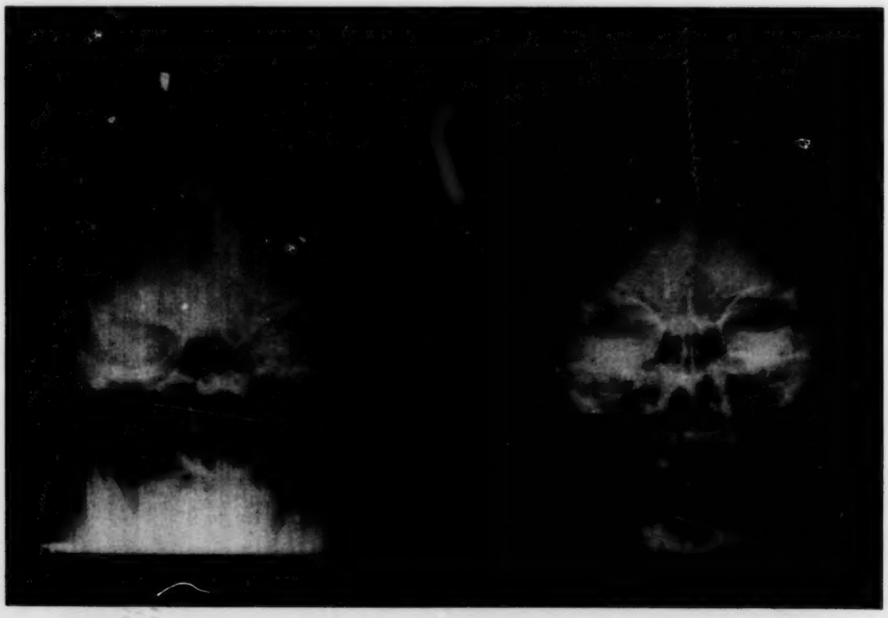

FIGURE 3.—Objective response after 10 mos of treatment with elliptinium (same patient as fig 2).

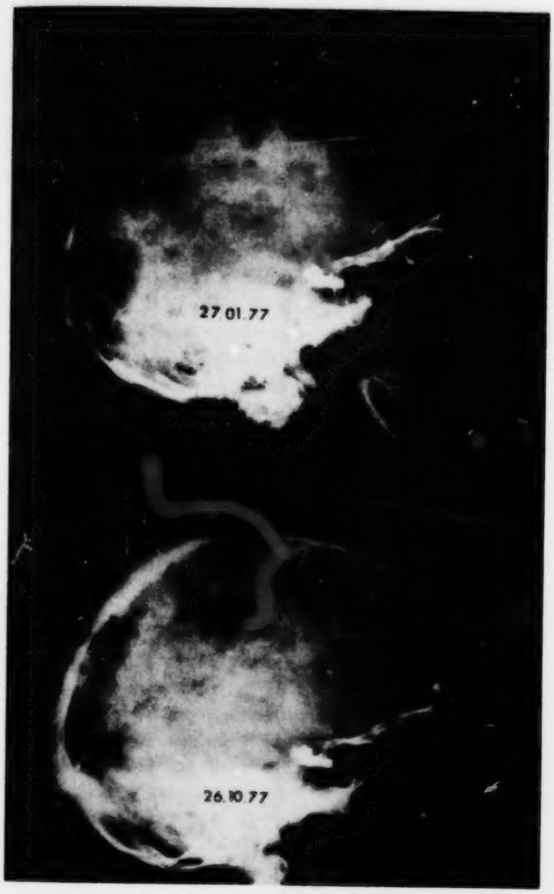

FIGURE 4.—Objective response after 9 mos of treatment with elliptinium (in the sella turcica are yttrium seeds and tantalum screw positioned during hypophysiolysis performed in Nov 1975).

mm^3 were able to undergo several months of treatment without aggravation of their marrow hypoplasia. This unusual tolerance can, however, only be affirmed under the conditions of our study, ie, at doses of 100 mg/m^2/week. Brugarolas et al (14) reported that patients receiving weekly doses of 100–160 mg/m^2 had a slight tendency to develop leukopenia and thrombocytopenia. Whatever it may be, the drug's lack of hematologic toxicity was found to be especially valuable in patients with osseous metastases from breast cancer, where frequent marrow involvement makes it difficult to administer the usual cytostatic agents.

Renal toxicity.—This complication was not reported in phase I studies of the drug (8,9). Irreversible renal insufficiency developed in four patients, whose treatment was continued after they presented abnormal creatinine levels, at respective cumulative doses of 2400, 2550, 2950, and 4000 mg/m^2. Biopsies in three of these patients showed that the renal insufficiency might be a consequence of tubular lesions; the glomeruli were intact. These renal disorders could not have resulted from hypercalcemia, a frequent complication of bone metastases, since the blood calcium level was normal in these four patients. Investigations are underway to determine whether a lower dose ($<$ 100 mg/m^2/week) combined with a fluid supplement might decrease these drug-induced nephrotoxic effects.

Asialia.—Paradoxically, dryness of the mouth, an apparently minor complication, often constituted the most serious obstacle to routine continuation of treatment. Three-fourths of the patients developed asialia after 6–8 weeks of treatment. This uncomfortable effect might also be the primary cause of other complications observed: oropharyngeal mycosis, anorexia, asthenia, weight loss, etc. The use of an effective artificial saliva (trials are currently underway) would considerably facilitate the continuation of treatment with elliptinium.

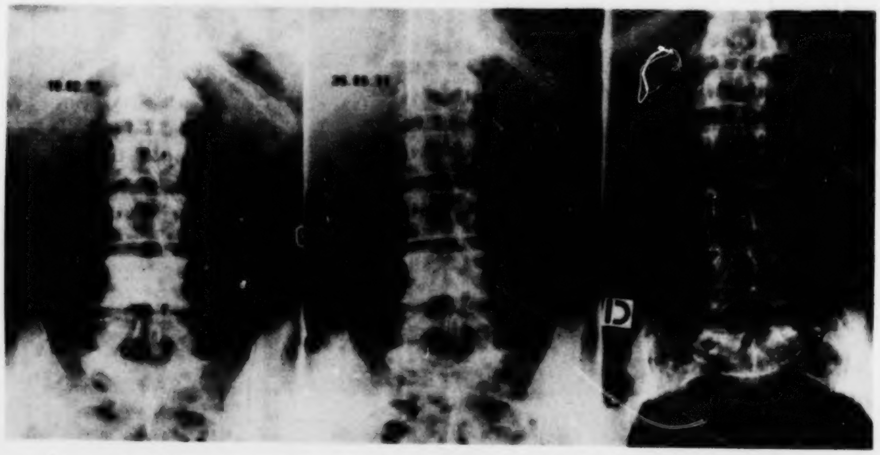

FIGURE 5.—Objective response after 4 mos of treatment in a patient with an osteoblastic metastasis.

DISCUSSION

The principal interest of elliptinium lies in the following two characteristics. (*a*) This compound is able to induce important remissions in bone metastases from breast cancer, and (*b*) its marrow toxicity (if any) is very low, with the therapeutic dosages used in this study. These characteristics are especially interesting since bone marrow involvement is frequent in cases of bone metastases and because the use of most chemical drugs is limited by their toxic effect on the bone marrow.

At the present time, the use of elliptinium in bone metastases from breast cancer raises the following two main problems.

(*a*) *What is the optimal dosage?*—In the first phase I studies (8,9), 80–100 mg/m^2/week appeared to be the most satisfactory dose. Since there was late nephrotoxicity, lower routine doses may be more suitable. With regard to the asialia, a trial is underway to see whether infusions on 2 consecutive days with intervals of 2 weeks, or on 3 consecutive days with intervals of 3 weeks, would obtain the same therapeutic effect with less unpleasant side effects.

(*b*) *What is the optimal place of elliptinium in the management of bone metastases from breast cancer?*—As stated earlier, first preference is given at the CFB to hormonal treatments, and chemical cytostatics are used after effects of the hormonal treatments wear off. Should elliptinium be given as the first chemical treatment, followed after failure by conventional cytostatics, or should the order be reversed? Our two nonrandomized series, comprising 66 and 69 patients, respectively, are too small to draw comparative conclusions. A randomized study has just been started in an attempt to compare the two protocols according to the number of remissions obtained, length of remissions, survival time, and discomfort of the patients.

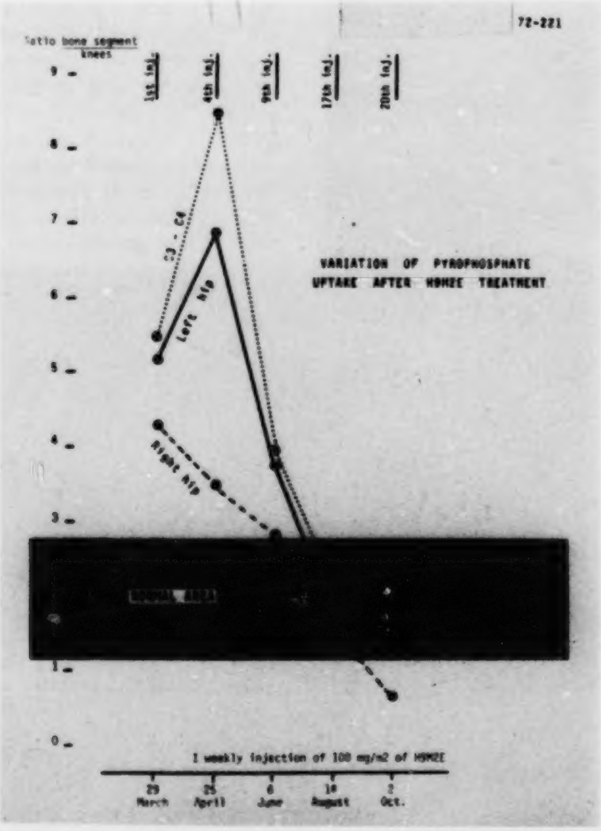

FIGURE 6.—Objective response after 8 mos of elliptinium, expressed by a normalization of the ^{99m}Tc pyrophosphate scintigram, quantified in terms of: $\frac{\text{uptake on metastatic segments}}{\text{uptake on normal bone}}$.

TABLE 3.—Toxic effects and intolerance disorders resulting from elliptinium (100 mg/m^2/wk) in 135 patients with bone metastases from breast cancer

Effects	No. of patients (%)	Comments
Digestive disorders		
Nausea	135 (100)	
Vomiting (usually slight)	103 (76)	In 7 patients, treatment had to be discontinued before the 6th infusion, because of severe vomiting
Marrow toxicity	0	In 99 patients with > 4000 wbcs/mm^3, no tendency toward leukopenia. In 36 patients with < 4000 wbcs/mm^3, no aggravation of the hypoplasia was observed. In 5 patients with < 1800 wbcs/mm^3 (platelets < 20,000/mm^3 in 2 cases), treatment could be continued from 7 to 29 infusions without worsening of these disorders
Liver toxicity	0	
Renal toxicity	8[*]	Irreversible insufficiency was observed in 4 patients who had received > 24 infusions
Immunologic disorders	35 (26)[†]	Hemolysis was observed in 3 cases; hemodialysis was necessary in 1 case
Asialia	101 (75)	
Tongue mycosis	10 (7)	
Fever	6 (4)	5 cases < 39°C, 1 case of 40°C, lasting not longer than 24 hrs
Cramps	32 (25)[‡]	Usually attenuated by quinine benzoate or amiodarone
Asthenia	[§]	
Anorexia	[§]	

[*] Rise in blood creatinine observed after 20 weekly infusions.

[†] Appearance of anti-ellipticine antibodies.

[‡] After 20 weekly infusions.

[§] Usual after 20 weekly infusions.

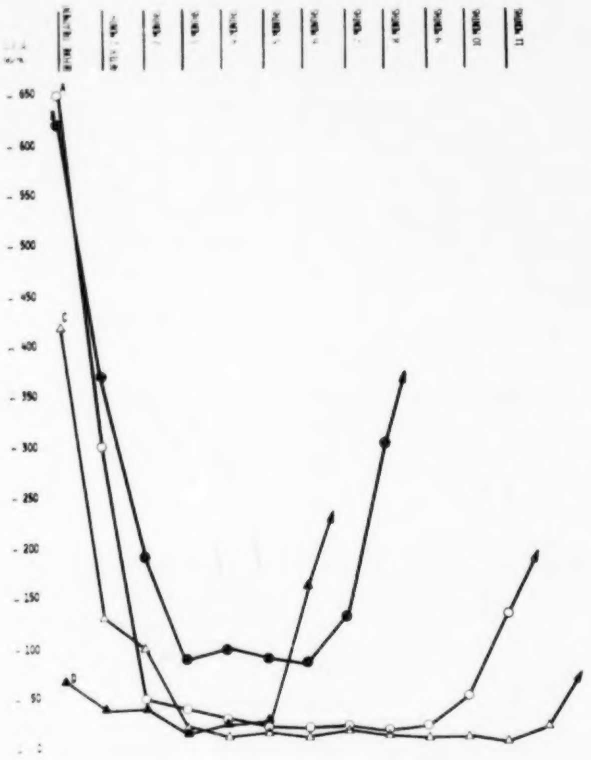

FIGURE 7.—Objective remissions expressed by the normalization of the serum CEA in 4 women with osseous metastases treated with elliptinium. Duration of remissions: 9 mos (patient A); 6 mos (patient B); 11 mos (patient C); and 5 mos (patient D).

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Results of Aggressive Chemotherapy for Acute Nonlymphoblastic Leukemia in Adults[1,2]

James O. Armitage,* C. Patrick Burns, Fred R. Dick, Suzanne B. Aunan, and Donald J. Slymen[3,4]

Sixty-five patients with acute nonlymphoblastic leukemia, ranging in age from 16 to 73 years (median, 54), were treated with thioguanine, cytarabine, daunorubicin, prednisone, and vincristine to induce complete remission. Remission was maintained with monthly 5-day courses of cytarabine and thioguanine for 4 years. Of the entire group, 57% achieved complete remission and 29% died in the first 30 days of treatment. The median remission duration was 15 months, with 20% of the complete responders predicted to remain in complete remission for 4 years. No primary central nervous system relapses have occurred. Of six variables examined by multivariate analysis, only age was predictive of remission success. Before analysis, the patients were divided into groups by age: *group 1*—ages 16–42 years ($n = 17$); *group 2*—ages 43–59 years ($n = 25$); and *group 3*—ages 60–73 years ($n = 23$). Complete remission rates were 76% in group 1, 64% in group 2, and 35% in group 3 ($P = 0.002$). Lower remission rates for older patients resulted from more early deaths rather than resistant disease. Age and LDH levels affected remission duration. The median complete remission duration was 48 months in group 1 and 10 months in groups 2 and 3 ($P = 0.004$). In group 1, 41% of patients achieving complete remission are predicted to remain in complete remission for 4 years. We conclude that this five-drug induction regimen is highly effective for induction therapy in younger adults with acute nonlymphoblastic leukemia. The results in patients ≥ 60 years of age are less satisfactory due to a lower remission rate and greater chance of early death. Furthermore, these results demonstrate that the program as described results in a longer duration of complete remission for patients of younger age. [Cancer Treat Rep 66:1917–1924, 1982]

Intensive combination chemotherapy in children with acute lymphoblastic leukemia has been successful in producing a cure in approximately one-half of the cases where it has been applied (1). Although similarly aggressive treatment in adults with acute nonlymphoblastic leukemia (ANLL) unfortunately has not met with the same success, recent reports have described improvement in the results of treating ANLL (2,3). Previous series have often contained a predominance of pediatric patients or had a younger median age than is characteristic for adult ANLL in general. Whether or not age is a prognostic factor in the treatment of ANLL has been a point of controversy. If older age is an adverse prognostic factor, it would be important to identify the reasons, and if possible, modify management accordingly. In addition, bone marrow transplantation after high-dose chemotherapy and total-body irradiation has recently been demonstrated to be effective "maintenance" or "consolidation" therapy in children and young adults with ANLL (4–6).

Since 1975, we have treated 65 adults with ANLL using an intensive remission-induction regimen of

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[1] Received Mar 25, 1982; revised July 23, 1982; accepted Aug 5, 1982.

[2] Supported in part by the Iowa Leukemia and Cancer Research Fund. Dr. Burns is the recipient of Public Health Service Research Career Development Award CA-00324 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Departments of Medicine (*J. O. Armitage, C. P. Burns*, and *S. B. Aunan*), Pathology (*F. R. Dick*), and Preventive Medicine and Environmental Health (Biostatistics Section) (*D. J. Slymen*), University of Iowa College of Medicine, Iowa City.

[4] We gratefully acknowledge the aid and support in patient care provided by the nurses and house staff of the University of Iowa Hospitals.

**Reprint requests to:* James O. Armitage, MD, Department of Medicine, University of Nebraska Medical Center, 42nd and Dewey Ave, Omaha, NE 68105.

thioguanine, cytarabine, daunorubicin, prednisone, and vincristine and then maintaining complete responses with monthly 5-day courses of cytarabine and thioguanine for 4 years. Preventive central nervous system (CNS) therapy was not given. In presenting the results of this treatment regimen, we describe the complete remission rate, the chances for long-term disease-free survival, the frequency of CNS relapse, and the effect of age and other prognostic factors on treatment outcome.

PATIENTS AND METHODS

Patient Characteristics

The patients in this study were first treated for ANLL at the University of Iowa Hospitals between February 1975 and June 1980. All patients treated with this regimen through June 1980 are included. The patients varied in age from 16 to 73 years (median, 54). The age limits of the three age groups used in analyzing the data (table 1) were established prior to any analysis. Sixty years has been frequently used in other studies. Patients < 60 years old were further divided at 42 years, since this is the age cutoff we have used for bone marrow transplantation at our institution. None of the patients had received previous antileukemia therapy. The subtype of acute leukemia and hematologic parameters for the patients are presented in table 1. ANLL was subclassified using French-American-British (FAB) criteria (7).

Complete remission was diagnosed when there were < 5% blasts or 10% blasts plus promyelocytes in a normally cellular marrow, along with a granulocyte count of at least 1.5 × 10^9/L, a platelet count of at least 100 × 10^9/L, and a rising hemoglobin level. Bone marrow was tested monthly before each course of maintenance chemotherapy, and relapse was diagnosed when the blasts were definitely > 5% or the blasts plus promyelocytes were > 10% of the marrow nucleated cells. Complete remission duration was defined as the time from diagnosis of complete remission to the identification of relapse, which was almost always when the patient was asymptomatic. Survival was defined as the time from beginning of chemotherapy to death. For purposes of this report, early death was defined as a death, almost always from infection, within 30 days of starting induction chemotherapy. The diagnosis of CNS leukemia was considered established when leukemic cells were identified in the cerebrospinal fluid. Routine cerebrospinal fluid examinations were not performed. However, lumbar punctures were performed whenever the clinical symptoms and signs were suggestive of CNS leukemia. Since August 1976, all cerebrospinal fluid examinations have included the evaluation of smears prepared using a cytocentrifuge technique.

TABLE 1.—Patient characteristics

Characteristic	Age group 1	Age group 2	Age group 3	All patients
Age (yrs)	16–42	43–59	60–73	16–73
No. of patients	17	25	23	65
Male:female	7:10	18:7	12:11	37:28
FAB subtype				
M1	4	6	7	17
M2	4	8	7	19
M3	2	1	1	4
M4	6	8	4	18
M5	1	1	4	6
M6	0	0	0	0
RAEB*	0	1	0	1
Wbc count (× 10^9/L)				
Median	26.6	8.5	3.8	
Mean	44.7	21.8	25.0	
Platelet count (× 10^9/L)				
Median	45.0	26.0	55.0	
Mean	114.9	55.5	88.7	
LDH level (IU/L)				
Median	606.0	472.0	318.0	
Mean	681.6	686.2	355.0	

*Diagnosed early in study as erythroleukemia then reclassified as refractory anemia with excess blasts (RAEB).

Treatment Regimen

The treatment regimen was as follows:

Induction*:	Thioguanine, 75 mg/m^2 orally q 12 hrs, Days 1–7 Cytarabine, 75 mg/m^2 iv q 12 hrs, Days 1–7 Daunorubicin, 60 mg/m^2 iv, Days 1–3 Prednisone, 40 mg/m^2 orally, Days 1–7 Vincristine, 1 mg/m^2 iv, Days 1 and 7
Consolidation:	Thioguanine, 75 mg/m^2 orally q 12 hrs, Days 1–5 Cytarabine, 75 mg/m^2 iv q 12 hrs, Days 1–5 Daunorubicin, 60 mg/m^2 iv, Days 1–2 Prednisone, 40 mg/m^2 orally, Days 1–5 Vincristine, 1 mg/m^2 iv, Day 1
CNS prophylaxis:	None
Maintenance:	Cytarabine, 100 mg/m^2 iv q 12 hrs × 5 days Thioguanine, 100 mg/m^2 orally q 12 hrs × 5 days, repeat q 4–5 wks

*Repeated on Day 15 if leukemia persisted in marrow.

All patients received the first 7-day sequence after pretreatment with allopurinol. A bone marrow biopsy was performed 7 or 8 days after completing the first 7 days of chemotherapy, and a treatment decision was based on marrow cellularity. If the marrow was hypocellular, further treatment was withheld until recovery occurred. If the marrow remained cellular (ie, $\geq 30\%$ cellularity) with persistent leukemia, the 7-day treatment sequence was repeated. In the very unusual case of a cellular marrow with normal maturation, treatment was withheld. As soon as the patient met the criteria for complete remission, the 5-day consolidation sequence utilizing the same agents was administered. Then, when marrow and peripheral blood recovery was evident, the maintenance regimen was begun and administered at 4- or 5-week intervals for 4 years. Blood cell counts were routinely performed only at the initiation of each maintenance sequence. There was no significant difference in dose adjustments among the different age groups. After 4 years of continuous remission, all treatment was discontinued and the patients were followed with intermittent bone marrow examination. Patients were uniformly hospitalized during the consolidation sequence. Maintenance chemotherapy was administered on an outpatient basis, with the iv cytarabine given in a clinic, emergency room, or, rarely, by a family member or neighbor. Post-relapse therapy was individualized.

Statistical Methods

All patients in this study were considered evaluable. Complete remission duration and survival of groups were calculated using the life table method. Factors influencing survival time and complete remission duration were analyzed using the proportional hazards model. The model may be stated in terms of the hazard function, $\lambda(t)$, which for survival time may be defined as the probability that an individual will die in a short time interval, given survival to the beginning of the interval. The hazard function may be expressed for an individual as:

$$\lambda(t) = \exp\{X'\beta\}_0(t),$$

where $\lambda_0(t)$ is the underlying hazard for all individuals, X is a p-vector of observed values on an individual for the set of p factors we wish to examine, and β is a p-vector of unknown regression coefficients which will be estimated. This method is detailed in the report of Cox (8); a simpler approach to the estimation scheme, which was employed in this analysis, was derived by Breslow (9).

The analysis of factors influencing achieving complete remission was accomplished using a multiple logistic model. It is assumed that the probability of achieving complete remission is $\exp\{-X'\beta\}/(1 + \exp\{-X'\beta\})$, where X is the vector of observed values and β is the vector of regression parameters to be estimated. Six variables were examined: age group (1 = 16–42, 2 = 43–59, and 3 = 60–73), wbc count, platelet count, serum LDH level, sex, and subtype of ANLL (combined M1, M2, and M3 versus combined M4 and M5). The one patient with RAEB (table 1) was excluded from this analysis. In addition, age without grouping was examined. Table 1 displays the mean and median wbc and platelet counts and LDH levels by age group. For the 64 patients included in the analyses, 13 had censored survival times. An equivalent sample size with no censoring is 58. Complete remission was achieved for 36 of the patients included in the analyses. Of these, 12 had censored remission duration times; an equivalent sample size with no censoring is 29.

Multivariate analyses examined the simultaneous effects of the set of risk factors on survival time, complete remission, and remission duration. For each analysis, a backward elimination procedure was used to remove from the model variables that did not contribute additional significant information to the model beyond the set of factors included. The variable with the smallest chi-square statistic was removed at each step, provided the associated P value was > 0.05. The analyses are reported using the age grouping factor instead of age. However, when the actual age values were used, the results from the backward elimination method were substantially the same. The significance of differences between groups that were not part of the multivariate analysis was determined using Fisher's exact test.

RESULTS

Thirty-seven of the 65 patients (57%) in this series achieved complete remission. The chances for complete

TABLE 2.—Results of treatment by age group

Characteristic	Age group 1	2	3	All patients
Complete remissions (CR)(%)*	76	64	35	57
Early deaths (ED)(%)†	6	24	52	29
CR + ED (%)	82	88	87	86
Median CR duration (mos)‡	48	10	10	15
CNS relapses	0	0	0	0
Predicted 4-yr CR (%)	41	8	17	20
Overall median survival (mos)§	27	9	< 1	9

*1 + 2 > 3, $P = 0.002$.
†1 < 2 + 3, $P = 0.015$.
‡1 > 2 + 3, $P = 0.004$.
§1 > 2 + 3, $P < 0.001$.

remission varied by age, being 76% in group 1, 64% in group 2, and 35% in group 3 (table 2). Multivariate analysis identified only age (ie, either by age group or individually) as significantly associated with chances to achieve a complete remission. Patients in groups 1 and 2 were significantly more likely to achieve complete remission than those in group 3 ($P = 0.002$). Sex, wbc count, platelet count, subtype of ANLL, and LDH level did not affect the chances of achieving a complete remission. The frequency of early deaths (ie, within 30 days of the beginning of therapy) and complete remission rate were inversely related, and the sum of the complete remission rate and the early death rate was relatively constant between groups. Therefore, any variation among age groups in the proportion of patients with chemotherapy-resistant disease could not explain the differences in outcome. Early deaths occurred in 6% of the patients in group 1, in 24% of the patients in group 2, and in 52% of the patients in group 3. Patients in groups 2 and 3 were significantly more likely to die in the first 30 days of therapy than those in group 1 ($P = 0.015$).

Twenty percent of the entire group of 37 patients achieving complete remission are predicted to remain in remission at 4 years, with a median complete remission duration of 15 months (fig 1). The duration of complete remission, once achieved, is presented by age group in figure 2. Eight of 13 complete responders in group 1, three of 16 in group 2, and two of eight in group 3 remain in complete remission from 5+ to 69+ months

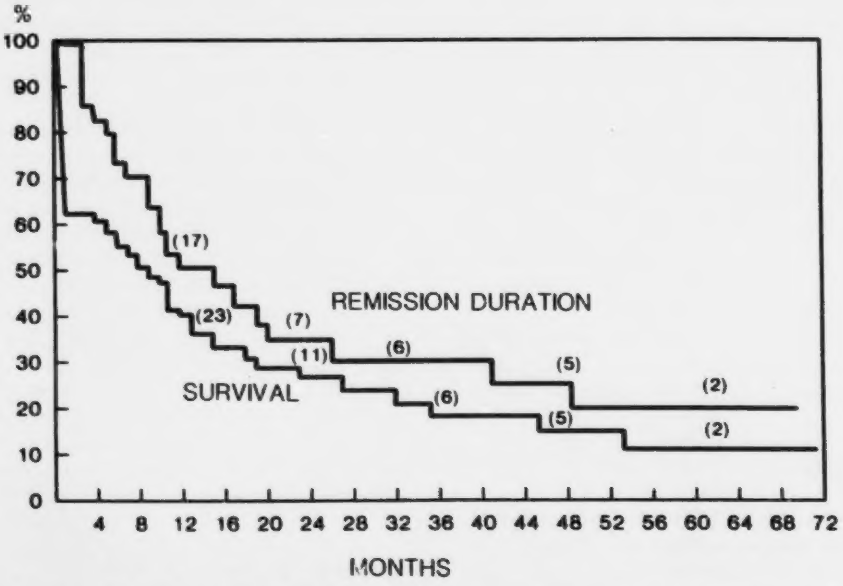

FIGURE 1.—Survival from initiation of therapy for the entire group of 65 patients, and complete remission duration for the 37 patients achieving remission. Values in parentheses = No. of patients surviving or remaining in remission to the indicated interval.

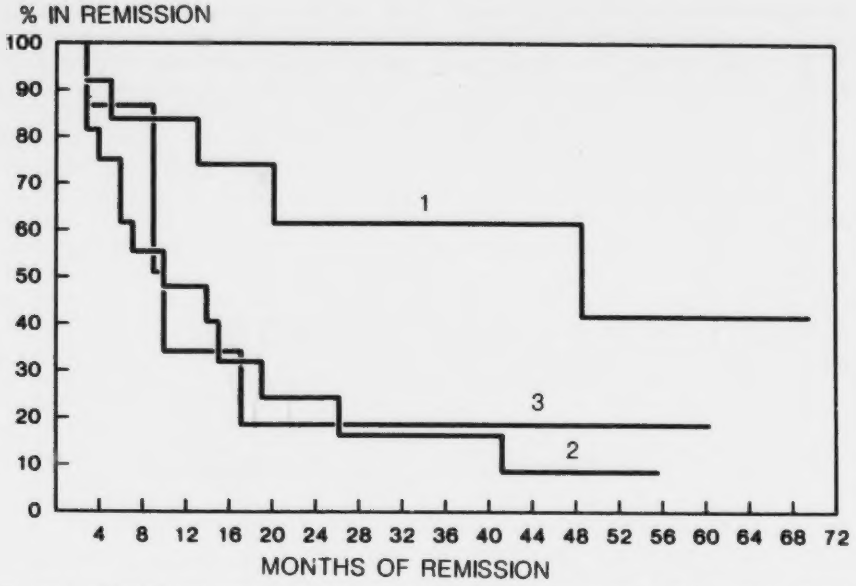

FIGURE 2.—Complete remission duration for patients in the following age groups: 16–42 (group 1, 13 patients), 43–59 (group 2, 16 patients), and 60–73 (group 3, 8 patients). Patients in group 1 had significantly longer remissions than patients in groups 2 and 3 ($P = 0.004$).

(median, 24+). The median complete remission duration was 48 months in group 1, 10 months in group 2, and 10 months in group 3 (table 2). Forty-one percent of patients in group 1 are predicted to be in complete remission at 4 years, in contrast to 8% of patients in group 2 and 17% of patients in group 3. Patients in group 1 had significantly longer remissions than those in groups 2 and 3 ($P = 0.004$). Multivariate analysis identified age group, age, and LDH level as being significantly associated with remission duration. Prolonged remissions were associated with lower levels of LDH ($P = 0.002$). The effects of age and LDH on remission duration were examined in another model and were independent of one another. In fact, younger patients, who had the longest remissions, also had the highest LDH levels ($P = 0.007$), and the effect of LDH on remission duration was evident within each age group. An effect of LDH on remission duration has been previously reported (10). Sex, wbc count, platelet count, subtype of ANLL, and peak SGOT level in the first 2 months of therapy (ie, to estimate the effect of treatment-related hepatitis) did not significantly affect remission duration. There were no primary CNS relapses despite the absence of prophylactic therapy to the CNS. To date, there have been no relapses in four patients followed 1, 8, 13, and 21 months after maintenance treatment was discontinued.

The median survival for the entire group of 65 patients is predicted to be 9 months (fig 1). This varied from 27 months in group 1, to 9 months in group 2, to < 1 month in group 3 (fig 3). Fourteen patients remain alive from 7 to 71 months after beginning therapy. Survival was significantly associated with age (ie, either by age group or individually) and LDH level. The survival of patients in group 1 was significantly longer than for patients in groups 2 and 3 ($P = 0.001$). Lower levels of LDH were associated with prolonged survival ($P = 0.002$). The effects of age and LDH on survival were examined in another model and were independent. Sex, wbc count, platelet count, and subtype of ANLL did not significantly affect survival.

DISCUSSION

In this study we found age-dependent remission rates varying from 76% to 35% and durations of remission from 48 to 10 months. The data demonstrate that the five-drug induction and consolidation program followed by the two-drug maintenance we utilized is effective in the therapy of adult ANLL. The fact that 41% of the younger patients achieving a remission are predicted to remain in remission for 4 years demonstrates that the disease can now be controlled and perhaps cured in an appreciable percentage of patients.

We selected the five-drug induction program for inclusion in our regimen after it was initially reported to have favorable results (11) and had showed promising results in our own preliminary experience as part of a

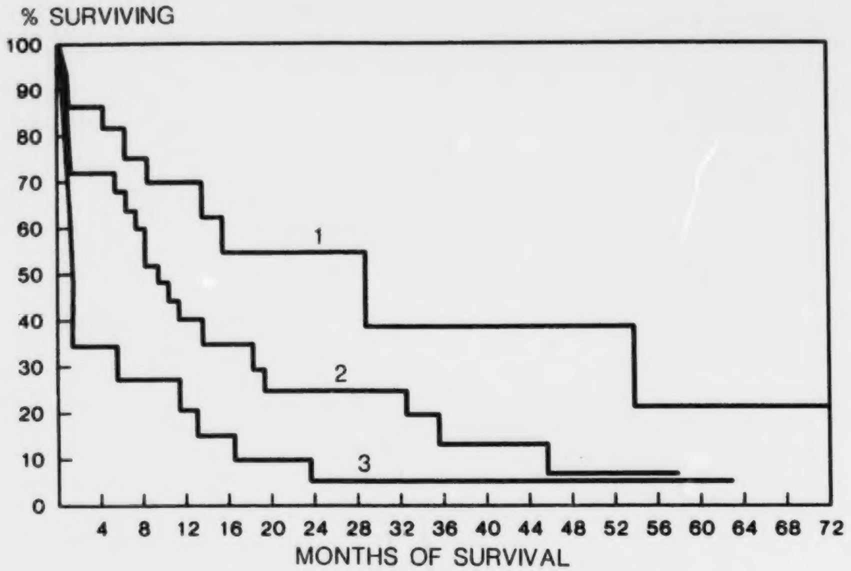

FIGURE 3.—Survival from the initiation of therapy for all treated patients in the following age groups: 16–42 (group 1, 17 patients), 43–59 (group 2, 25 patients), and 60–73 (group 3, 23 patients). Patients in group 1 survived significantly longer than those in groups 2 and 3 ($P < 0.001$).

multi-institutional trial (12). Furthermore, the combination seemed theoretically logical. It seemed clear that daunorubicin and cytarabine, which are the two most active single agents for adult ANLL, should be included in induction programs. The addition of thioguanine seemed justified on the basis of studies in animal models which showed it to be synergistic with cytarabine in experimental systems (13). Vincristine and prednisone have some small single-agent activity (14). We were attracted to a program which included them because of the occasional impreciseness of the differentiation between ANLL and ALL in adults. For example, the L2 lymphoid category of the FAB classification has been considered as undifferentiated or stem cell leukemia and treated as ANLL by many institutions; in one recent series, one-half of these cases exhibited high levels of terminal transferase and a lower remission rate when compared to other myeloid groups (15). The maintenance program was utilized because of our previously reported good results (16) and the desirability of including a drug which crosses into the CNS when administered systemically.

The adverse effect of age on chances to achieve a complete remission from ANLL with chemotherapy has, with a few exceptions (17,18), been a reproducible finding in previous studies. In the present study, age had no statistically significant effect until 60 years. The relatively constant sum of complete remission rate and early death rate in the various age groups in this study suggests that the lower remission rate in older patients is a direct result of their inability to tolerate the period of hypoplasia that is associated with remission induction chemotherapy. Thus, shortening the period of hypoplasia by a modification in therapy or improved supportive measures would seem obvious approaches to this problem. It may be that the older group of patients are the ones most likely to benefit from protected environments such as laminar air flow rooms or prophylactic antibiotics.

Some previous studies have reported that there is no adverse effect of age on duration of remission (19–23). In our study, there was a significantly longer duration of remission for patients < 42 years old compared to those > 42. However, the age groups 43–59 and 60–73 did not differ. Other studies have also reported that duration of remission is inversely related to age (10,24).

Although CNS involvement in adults with ANLL is certainly much less frequent than in children with acute lymphoblastic leukemia, it has been increasingly recognized as a clinical problem and seems to be particularly associated with monocytic subtypes (25). As complete remission durations improve, it might be anticipated that CNS relapse would become more frequent and necessitate attempts at prophylactic CNS therapy. With the treatment regimen we employed, this has not been the case. Meyer et al (25) and Wiernik et al (26) have demonstrated that routine lumbar punctures reveal unexpected CNS leukemia in as many as

18% of newly diagnosed patients. Because routine lumbar punctures were not performed in this study, it is impossible to be certain that subclinical disease was not present in a proportion of patients. However, the absence in all patients of overt CNS leukemia terminating remission makes this unlikely. The low CNS relapse rate that we observed may be related to the inclusion of cytarabine in the maintenance therapy. Cytarabine is known to penetrate the CNS (27), and Herman et al (28) observed a decreased incidence of CNS recurrence in patients with diffuse histiocytic lymphomas when a maintenance regimen including cytarabine was used.

The maintenance treatment that we employed is administered on an outpatient basis and is associated with minimal morbidity and no mortality (16). Similar results using a comparable maintenance regimen have been reported by Peterson and Bloomfield (19). More intensive maintenance regimens and "late intensification" requiring hospitalization and associated with morbidity and risk of death should not be accepted as standard approaches until it is demonstrated that they yield superior survival to effective outpatient maintenance programs.

It is not possible to know whether the successful outcome of our therapeutic program is the result of tumor burden reduction during induction, consolidation, or maintenance. However, there are data to suggest that the maintenance portion is an important component. Glucksberg et al (29) have recently reported remission rates similar to ours using an induction and consolidation program that is identical to ours in drugs and schedule and differing only slightly in doses. In that study, the median remission duration of 39 weeks was considerably less than the 64 weeks in our study. If this difference is real, as it appears to be, the longer duration in our study could have resulted from a more effective maintenance program which differed from theirs in the dose and rate of cytarabine and because they also utilized prednisone and vincristine. Furthermore, for most of their patients maintenance therapy was discontinued at 30 months.

Several recent studies besides our own have demonstrated improved results in the chemotherapy of ANLL (3,19,29–31). Complete remission rates as high as 85% (32) and median remission durations with chemotherapy for all patients of as long as 27 months (33) have been reported. It has become apparent that a proportion of patients can have long-term disease-free survival after chemotherapy has been discontinued (30). A small but real proportion of adults with ANLL can be cured using currently available chemotherapy. Bone marrow transplantation after high-dose chemotherapy and total-body irradiation when applied to patients with ANLL in remission has been reported to yield relapse-free survival in > 60% of patients with observation as long as 3 years (4). However, bone marrow transplantation is limited by age and by the fact that approximately 60% of potential candidates lack a donor. Children tolerate bone marrow transplantation better than adults, and few successful transplants have been performed after the age of 40. Whether a similar effect of age on survival in complete remission is operative in patients treated with maintenance chemotherapy has been a point of controversy, as previously discussed. In this study, we found a long remission duration in adults in the age group that our institution has considered candidates for bone marrow transplantation. These results emphasize the need for a prospective clinical trial comparing bone marrow transplantation with an effective maintenance chemotherapy regimen in young adults.

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Use of Methotrexate and 5-FU for Recurrent Head and Neck Cancer[1]

Charlotte Jacobs[2,3,*]

Thirty patients with recurrent squamous cell carcinoma of the head and neck were treated with methotrexate (250 mg/m^2) followed 1 hour later by 5-FU (600 mg/m^2). One patient had a complete response and four had partial responses, for an overall response rate of 16%. The response rate in patients with no prior chemotherapy was 21%. Toxic effects included neutropenia in ten patients, with one associated death from infection; severe mucositis in three patients; and severe diarrhea in seven patients. Three patients had acute abdominal pain requiring hospitalization, with one associated death. The combination of methotrexate and 5-FU as given in our study was not superior to methotrexate alone and had substantial toxicity. [Cancer Treat Rep 66:1925–1928, 1982]

The role of chemotherapy in the management of recurrent squamous cell cancers of the head and neck has been limited (1–3). Methotrexate, the most extensively studied drug, has reported response rates from 8% to 50%, but most responses are partial and of short duration (4). Although combination chemotherapy has been reported to have higher response rates (5–7), there is often associated increased toxicity. Only one randomized study has shown improvement in response rates with a combination of agents (cisplatin, methotrexate, and bleomycin) compared to methotrexate alone (8).

Pitman et al (9) have described a highly effective regimen of sequential methotrexate plus 5-FU in which treated patients with squamous cell cancer of the head and neck had a 40% complete response rate. This regimen was based on laboratory studies by Cadman et al (10) in which they showed that exposure of L1210 leukemia cells first to methotrexate and then to 5-FU produced a synergistic effect on cell kill.

The purpose of this study was to investigate further the efficacy and toxicity of this regimen for recurrent squamous cell cancers of the head and neck.

PATIENT SELECTION AND METHODS

Between July 1980 and May 1982, 30 patients with recurrent squamous cell carcinoma of the head and neck were treated with moderate-dose methotrexate and 5-FU. All patients had recurrent disease following surgery and/or radiation therapy, and 16 patients had had prior chemotherapy, predominantly with single-agent cisplatin.

Pretreatment evaluation included a complete history and physical examination, cbc, serum creatinine, urinalysis, BUN, liver enzymes, chest X-ray, and appropriate radiographs and scintiscans for measurement of disease. Cbc and serum creatinine were measured prior to each treatment.

Methotrexate (250 mg/m^2) was infused over 30 minutes and was followed 1 hour later by 5-FU (600 mg/m^2). The patient was prehydrated with 1 L of 5% dextrose in water plus 100 mEq of sodium bicarbonate, and 1 L of the same iv fluid was infused over 2–4 hours following methotrexate. At 24 hours, patients began leucovorin orally: 25 mg initially, then 10 mg every 6 hours for six doses.

Response was evaluated prior to each treatment. A complete response was defined as the complete disappearance of all clinically detectable disease. A partial response was defined as a 50% reduction in the sum of the products of the longest diameters of all measurable lesions without the appearance of new lesions. Duration of response was calculated from the start of response to disease progression. Survival time was calculated from study entry to death. Toxicity was graded according to Northern California Oncology Group Toxicity criteria.

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[1] Received June 14, 1982; accepted Aug 24, 1982.

[2] Division of Oncology, Department of Medicine, Stanford University School of Medicine, CA.

[3] I thank Drs. Peter Eisenberg, George Waltuch, and Michael Alexander for clinical material.

[*] *Reprint requests to:* Charlotte Jacobs, MD, Division of Oncology, Department of Medicine, Stanford University Medical Center, Rm C005, Stanford, CA 94305.

RESULTS

Patient Characteristics

There were 21 males and nine females, with a mean age of 56 years (27–76). The mean Karnofsky performance status was 80 (range, 40–100). The primary sites of disease were as follows: oropharynx, nine patients; oral cavity, six; larynx, five; nasopharynx, four; hypopharynx, three; sinuses, two; and unknown primary, one. Twenty-three patients had local disease recurrence, five had distant metastases, and two had local disease plus distal metastases. All patients had had prior treatment: 20 had had surgery plus radiation therapy, ten had received radiation therapy, and 16 had had chemotherapy. All patients had squamous cell carcinoma, and the histologic differentiation was determined in 26: moderately differentiated, 12 patients; poorly differentiated, seven; well-differentiated, six; and undifferentiated, one. The mean number of treatments was five (range, one to 15), delivered over 9 weeks. All patients received the full calculated dose at each treatment, but treatment interval varied. Most patients received three weekly treatments, followed by treatments every other week.

Therapeutic Results

Of the 30 patients, one achieved a complete response (3%) lasting 19+ months and four achieved partial responses (13%) lasting 4+, 5, 5, and 7+ months. Thus, the overall response rate for the entire group was 16% (table 1). There was no significant difference in response rate between patients who had received prior chemotherapy (13%) and those who had had no prior chemotherapy (21%).

There was no difference in the following prognostic variables between patients who responded and nonresponders: age, sex, primary site, and site of disease recurrence. No patients responded to treatment who had a Karnofsky status < 80 or who had a poorly differentiated or undifferentiated cancer.

The mean survival for the entire group was 4 months (1–23+). The mean survival was 8 months for the responders and 3 months for the nonresponders.

Toxicity

All patients were evaluable for toxicity (table 2). Ten patients developed neutropenia with a wbc count $< 3000/\text{mm}^3$, associated with one episode of aspiration pneumonia and death. Two patients had platelet counts $< 100{,}000/\text{mm}^3$, and one had local bleeding at the base of the tongue.

Seven patients developed mucositis; it was severe in three. Three patients had moderate to severe vomiting. Seven patients developed diarrhea, four requiring hospitalization. Three of the hospitalized patients had associated severe abdominal pain; one died with pseudomembranous colitis. One patient underwent laparotomy for suspected appendicitis; no lesion was found.

No patient developed a creatinine elevation > 1.5 mg/100 ml. One patient developed severe dermatitis. Seven patients had treatment discontinued because of toxicity: two had recurrent severe mucositis; one had severe dermatitis; three had severe diarrhea and abdominal pain; and one patient died of infection.

DISCUSSION

Chemotherapy has been only moderately effective in palliating recurrent squamous cancers of the head and neck (2,3). The most effective agents include methotrexate, cisplatin, bleomycin, and 5-FU (1,3,4), but response rates are low, and most responses are partial and of short duration. The goal of combination chemotherapy is to increase complete responses and survival without substantial toxicity. Multiple combinations have been developed but none achieve these goals better than single agents (8,11,12).

An innovative treatment of sequential methotrexate and 5-FU was developed by Pitman et al (9) based on studies by Cadman et al (10) showing synergistic tumor cell kill in vivo in mice with L1210 leukemia and in vitro. Since in vitro methotrexate concentrations $> 10\ \mu\text{M}$ were necessary to maximize the synergy, Pitman et al administered methotrexate at doses of 125–250 mg/m^2. This was followed 1 hour later by 5-FU (600 mg/m^2 iv). Leucovorin rescue was begun at 24 hours at a dose of 10 mg/m^2 every 6 hours for six doses. The regimen was given weekly to toxicity and then every 2 weeks. At

TABLE 1.—Results of treatment

	No. of patients	No. of— Complete responses	Partial responses	Overall responses
No prior treatment	14	0	3	3
Prior treatment	16	1	1	2
Total	30	1	4	5

TABLE 2.—Toxicity

	No. of patients
Total	30
Hematologic (cells/mm^3)	
Wbc count < 3000	10
Platelet count < 100,000	2
Gastrointestinal	
Mucositis*	
Grade 3	3
Vomiting†	
Grade 3	1
Grade 4	2
Diarrhea‡	
Grade 3	3
Grade 4	4

*Grade 3: confluent mucositis.

†Grade 3: 1–3 times daily. Grade 4: continuous, requires hospitalization.

‡Grade 3: ≥ 4 movements/day. Grade 4: requires hospitalization.

the first report ten patients were evaluable and all achieved a response (> 50%); four had complete responses.

We have used this regimen on 30 patients, with only a 16% response (> 50%) rate; one complete response lasted 21+ months. There are several possible explanations for the wide difference in responses. The first relates to differences in patient population. Response rates to chemotherapy for head and neck cancer are higher in patients who have had no prior treatment (13). Almost one-half of the patients in the study of Pitman et al (9) had had no prior treatment, although the investigators did not find that this factor influenced response. All of our patients had had prior surgery and/or radiation. In addition, more than one-half of our patients had had prior chemotherapy. However, the response rate in those 14 patients who had had no prior chemotherapy was still only 21%. Another important prognostic variable is Karnofsky status (14); our mean was 80. However, the response rate for those patients with a Karnofsky status ≥ 80 was still only 25%. Other factors in our patient population that may influence response, such as primary site (4), site of recurrence, and histologic differentiation (15), cannot be compared to those in the patients of Pitman et al (9).

A second reason for difference in response may relate to treatment. Our doses of chemotherapy were similar to those of the Yale group. The mean number of treatments per patient was five, compared to 7.7 in the patients of Pitman et al (9). Seven of our patients received only one or two treatments because of rapid progression or severe toxic effects. Pitman et al noted that all responses occurred by the sixth week. Our response rate was still only 29% in those 17 patients who received at least 6 weeks of treatment.

The toxic effects were similar, except that our patients had more severe neutropenia. This was significant in only one patient. The most toxic side effect in our series was severe diarrhea, which occurred in 23% of the patients. It was associated with acute abdominal pain in three patients after six, eight, and 12 treatments; there was one death. Bertino (personal communication) has suggested that this toxicity may be reduced by three weekly treatments, followed by treatments every 2 weeks.

In summary, the combination of methotrexate plus 5-FU at our institution produced durable responses (4+ to 19+ months), but the number of responses was low. We cannot explain why this is so different from the Yale series. We cannot recommend this regimen for general use until other groups confirm the initially reported high response rate or it is shown to be superior to methotrexate alone in a randomized trial.

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Phase II Trial of Mitoxantrone[1]

Kenneth C. Anderson,* Gary I. Cohen, and Marc B. Garnick[2]

Systematic substitutions on the basic anthraquinone nucleus have produced mitoxantrone (DHAD) (1), a compound with significant antitumor activity in animal tumor systems (2), which produces DNA single-strand breaks and high relative cytotoxicity as noted with doxorubicin (ADR) (3). Moreover, DHAD was shown to be less cardiotoxic in the rat model (4), in beagles, and in cynomolgus monkeys (5). Hepatobiliary excretion constitutes the main route of drug elimination, with a half-life of 112.8 minutes (6). Phase I–II studies (7–14) indicated that the dose-limiting toxicity was leukopenia. A phase II study of DHAD was undertaken using a starting dose of 12 mg/m^2 administered iv over 1 hour every 21 days.

MATERIALS AND METHODS

All patients had histologic confirmation of refractory cancer, were ambulatory, and had normal hematologic, renal, cardiac, and hepatic functions. Measurable disease and a life expectancy of at least 2 months were required. Prior treatment with ADR ($\leq$ 450 mg/m^2) did not exclude patients from this study. A starting iv dose of 12 mg/m^2 was administered by dilution of DHAD in 200 ml of saline infused over 60 minutes every 21 days, with the dose modifications shown in table 1.

Evaluation for response and toxicity usually required multiple DHAD treatments and observation for at least 21 days thereafter.

TABLE 1.—Criteria for dose modification

Granulocyte count nadir (cells/mm^3)	Platelet count nadir (cells/mm^3)	Total bilirubin (mg/dl)	Dose adjustment
> 2000	> 150,000	< 1.5	Increase by 1 mg/m^2
1000–2000	75,000–150,000	< 1.5	Maintain same dose
< 1000	< 75,000	1.5–3.0	Decrease by 1 mg/m^2

RESULTS

Seventy-seven patients (190 courses) were evaluable for response and toxicity (table 2). Ninety percent of the patients had received prior therapy: combination chemotherapy (43%), radiation therapy (7%), or both (40%). Twenty-seven patients had had prior ADR exposure; ADR had been discontinued in 23 (85%) because of progressive disease, while four (15%) had previously responded to ADR either as a single agent or in combination programs. Sixty of the 77 patients received more than one course of DHAD therapy; dose escalation was achieved in 22 patients. Of 13 patients who developed leukopenia requiring dose reduction, seven had evidence of cumulative myelosuppression.

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Entered	86*
Evaluable	77†
Male/female	47/30
Median age in yrs (range): 57 (22–78)	
Prior treatment	
Chemotherapy	33
ADR	27‡
Radiotherapy	5
Chemotherapy and radiotherapy	31
No prior therapy	8

*9 patients inevaluable due to inappropriate (low) dose modifications.
†Total No. of courses = 190.
‡Median dose = 400 mg/m^2; range, 60–500 mg/m^2.

Toxicity (Table 3)

Thirteen of 190 courses (7%) were associated with severe myelosuppression (granulocyte count nadir < 1000 mm^3) at a median of 11 days following drug

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[1] Received Apr 28, 1982; revised July 30, 1982; accepted Aug 16, 1982.

[2] Division of Medicine, Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA.

**Reprint requests to:* Kenneth C. Anderson, MD, Division of Medicine, Sidney Farber Cancer Institute, Harvard Medical School, 44 Binney St, Boston, MA 02115.

TABLE 3.—Toxic effects

Toxic effect	No. of patients	% of patients (n = 77)	% of courses (n = 190)
Nausea/vomiting	2	3	2
Phlebitis	1	1	1
Rash	1	1	1
Granulocyte count nadir (cells/mm^3)[*]			
$<$ 1000	13	17	7
1000–1999	30	39	17
2000–3000	22	29	29
Platelet count nadir $<$ 75,000 cells/mm^3[†]	4	5	2
Clinical hemorrhage	0	0	0
Sepsis/death[‡]	2	3	1
Cardiotoxicity	0	0	0

[*] 65 of 77 patients (84%) ultimately developed granulocyte count nadirs $<$ 3000/mm^3.

[†] No clinical hemorrhage.

[‡] 71-year-old female who had received prior radiotherapy and methyl-GAG and vinblastine chemotherapy for metastatic renal cancer had a granulocyte count of 400/mm^3 3 days after 3rd 12-mg/m^2 dose of DHAD and died of staphylococcal sepsis in spite of antibiotic therapy. 46-year-old male with colorectal cancer metastatic to lung and liver, who had received prior 5-FU therapy, received 12- and 13-mg/m^2 doses of DHAD and had a granulocyte count nadir $>$ 2000/mm^3. He developed a granulocyte count of 1000/mm^3 4 days after a 14-mg/m^2 dose of DHAD and died of gram-negative sepsis in spite of antibiotic therapy.

administration. In 65 of 77 patients (84%), 102 of 190 courses (53%) resulted in myelosuppression (granulocyte count nadir $<$ 3000/mm^3). Thrombocytopenia (platelet count nadir $<$ 75,000/mm^3) occurred in 2% of the courses, but platelet transfusions were not required. Nausea and vomiting were infrequent. Phlebitis at the site of drug infusion occurred only once and resolved promptly with hydrocortisone therapy. One patient developed a diffuse pruritic erythematous maculopapular eruption, which resolved promptly on discontinuation of the drug. None of the patients had clinical or electrocardiographic evidence of cardiac dysfunction, including 27 patients who had received prior ADR chemotherapy.

Response to DHAD Therapy (Table 4)

No patient achieved a complete response. Well-documented partial response was achieved by five patients with the following tumors (table 5): lung adenocarcinoma (two patients), breast carcinoma (one), colorectal carcinoma (one), and melanoma (one). Four patients who had colorectal carcinoma and rapidly progressing liver metastases before DHAD treatment had either disease stabilization (cessation of progression) or $<$ 50% regression of measurable lesions for 1–2.5 months after DHAD treatment. Sixty-eight of the 77 patients (88%) had clear-cut disease progression occurring after two or three courses of DHAD. This included three of

TABLE 4.—Therapeutic response to DHAD

	No. of patients (%)				
Diagnosis	Total	Partial response	Stable disease	Progressive disease	Prior ADR therapy
Non-oat cell lung cancer	17	2[*] (12)	0 (0)	15 (88)	13 (76)
Renal cancer	10	0 (0)	0 (0)	10 (100)	1 (90)
Breast cancer	4	1[*] (25)	0 (0)	3 (75)	4 (100)
Melanoma	7	1 (14)	0 (0)	6 (86)	0 (0)
Colorectal cancer	29	1[†] (3)	4 (14)	24 (86)	0 (0)
Sarcoma	4	0 (0)	0 (0)	4 (100)	4 (100)
Other	6	0 (0)	0 (0)	6 (100)	5 (83)
Total	77	5 (7)	4 (5)	68 (88)	27 (35)

[*] 1 patient with prior ADR therapy.

[†] 1 patient with prior 5-FU/PALA therapy.

TABLE 5.—Partial responders to DHAD

Age	Sex	Diagnosis	Prior therapy*	Total dose of DHAD (mg/m²)	Response	Response duration (mos)
61	M	Non-oat cell lung cancer	None	60	> 50% ↓, lung mass; CEA, 15 ← 3 ng/ml	4.0
72	M	Non-oat cell lung cancer	C, ADR, P, XRT	36	> 50% ↓ hilar mass; CEA, 270 ← 130 ng/ml	4.5
54	F	Breast cancer	BS + O, C, M, 5-FU, ADR	96	> 50% ↓ bony metastases; CEA, 170 ← 85 ng/ml	2.0
75	M	Colorectal cancer	5-FU, PALA	73	> 50% ↓ hepatic metastases	4.0
72	M	Melanoma	None	34	> 50% ↓ pulmonary nodules	1.5

*C = cyclophosphamide; P = cisplatin; XRT = radiation therapy; BS + O = bilateral salpingo-oophorectomy; and M = methotrexate.

four patients with breast cancer who had previously responded to ADR therapy.

DISCUSSION

The data of this phase II study in selected solid neoplasms suggest that DHAD has activity against non-oat cell lung cancer but not colorectal cancer. Inadequate numbers of patients with melanoma and breast cancer make any conclusions premature. An overall objective partial response was observed in five of 77 evaluable patients (7%). The observed partial response rate in patients with non-oat cell lung carcinoma is 12% (two of 17 patients), with a ≥ 90% probability that the observed response will be 0–20%. One of these patients and the responding patient with breast carcinoma both had disease progression after receiving 450 mg/m² of ADR. The response rate of 3% in 29 patients with colorectal carcinoma assures with a ≥ 90% probability that the true response rate is ≤ 5%.

The dose-limiting toxic effect in all phase I–II studies of patients treated with DHAD has been leukopenia (7–14), as was true in our study. Although cardiotoxicity after DHAD treatment has been documented in nine patients previously treated with ADR (L. Lev, personal communication, Oct 1981), 27 patients in our study who had been previously treated with ADR manifested no cardiotoxicity as monitored by clinical assessment and electrocardiography.

In summary, this study confirms the mild toxicity of DHAD administered in a 12-mg/m² iv bolus dose every 3 weeks. Partial responses were documented in non-oat cell lung cancer, breast cancer, colorectal cancer, and melanoma. Prior therapy with ADR did not predispose to resistance to DHAD. Although no cardiotoxicity was observed in this study, it is premature to definitively conclude that prior ADR therapy does not predispose to DHAD-related cardiotoxicity.

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Cisplatin Urinary Pharmacokinetics and Nephrotoxicity: A Common Circadian Mechanism[1,2]

Francis Levi, William J. M. Hrushesky,* Richard F. Borch, Michael E. Pleasants, B. J. Kennedy, and Franz Halberg[3]

Renal toxicity and urinary pharmacokinetics following a nonlethal dose (5 mg/kg) of cisplatin were investigated in female F344 (Fischer) rats that were on a standardized light-dark schedule, with water freely available. Circadian timing of drug administration had a major effect on the BUN increase ($P < 0.01$), the urine volume increase ($P < 0.025$), and the urinary concentration of cisplatin following drug administration ($P < 0.01$). Renal toxicity was positively correlated with the peak urinary concentration of cisplatin and with the area under the curve of urinary concentration over the first 20 hours after drug administration ($P < 0.01$). Drug administration near the normal circadian maximum of urinary volume prior to treatment resulted in the least renal toxicity, lowest peak, and smallest area of urinary concentration of cisplatin. [Cancer Treat Rep 66:1933–1938, 1982]

Cisplatin is an important anticancer drug that has exhibited activity against a wide variety of human malignancies (6,25). Its dose-limiting side effect is irreversible nephrotoxicity (4,22), and the microscopic finding of tubular necrosis is common following cisplatin administration (8,18). It has been suggested that the urinary concentration of cisplatin may determine the amount of cisplatin-induced renal damage (3,9). This hypothesis is supported by the fact that aggressive hydration protocols reduce the nephrotoxicity of this drug (2,16,24).

Renal function is very rhythmic on the circadian time scale (5,15,26–28). The total extent of predictable circadian variation of most urinary variables investigated in rodents and human beings ranges between 100% and 200% of the 24-hour mean (11), and these circadian rhythms in renal function persist under various environmental conditions.

In previous murine work (10,17,20), we have demonstrated that a large-amplitude circadian rhythm characterizes renal tolerance for a high dose of cisplatin (11 mg/kg). Optimal renal tolerance for this drug occurs very near the time of day associated with the normal circadian maximum in urinary volume. The protective effect of concurrent hydration is largest when both cisplatin and hydration are given at this optimal time. Conversely, when both agents are administered near the time of day associated with the circadian minimum in urinary volume, little, if any, renal protection results from hydration. The studies reported here investigate whether a circadian rhythm in renal tolerance for a nonlethal dose of cisplatin exists in two different strains of rats (Fischer and Louvain) and whether the urinary pharmacokinetics of this drug differ significantly as a function of circadian timing of cisplatin administration.

MATERIALS AND METHODS

Study Designs

Studies 1 and 2.—Study 1 (May 28, 1978) and study 2 (Sept 21, 1978) were performed with a total of 23 singly housed female F344 (Fischer) rats, 2–6 months of age and weighing 90–180 g. These animals were standardized to a schedule of 8 hours of light alternating

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[1] Received Nov 2, 1981; revised July 13, 1982; accepted Aug 9, 1982.

[2] Supported by Public Health Service grants GM-13981 from the National Institute of General Medical Sciences, CA-31635 and CA-14445 from the National Cancer Institute, AG-00158 from the National Institute on Aging (National Institutes of Health), and OH-00631 from the National Institute of Occupational Safety and Health, Department of Health and Human Services; and by grants from the Masonic Memorial Hospital Fund, Inc; the Minnesota Medical Foundation; the American Cancer Society; and the St. Louis Park Medical Center Research Foundation (#298-81). Dr. Hrushesky is an American Cancer Society junior faculty fellow (grant 509A).

[3] Section of Medical Oncology, Department of Medicine, (*F. Levi, W. J. M. Hrushesky*, and *B. J. Kennedy*), Department of Chemistry (*R. F. Borch* and *M. E. Pleasants*), and Chronobiology Laboratories (*F. Halberg*), University of Minnesota Medical School, Minneapolis.

**Reprint requests to:* Dr. William Hrushesky, Section of Medical Oncology, Department of Medicine, PO Box 286, University of Minnesota Hospitals, Minneapolis, MN 55455.

with 16 hours of darkness for 3 weeks prior to and throughout the study. In order that food not contaminate urine specimens, food availability was progressively restricted to the first 2 hours of darkness. Fourteen rats were kept in plastic cages, and nine rats were housed in metabolic cages. For 10 days before and 6 days after drug administration, urine samples were obtained every 4 hours (using an automatic urine collector) from the animals kept in metabolic cages. Two times were selected for cisplatin injection: that of the maximum (acrophase) and that of the minimum (bathyphase) of the mean circadian rhythm in urine volume assessed before drug administration. The body weight of each rat was measured daily at 8 hours after light onset (8 HALO) during the 6-day span following drug injection.

Isolation of animals was necessary to obtain accurate measurements of urine volume from individual rats. The effect of isolation on rats must be considered, but this variable was kept constant across all groups.

Study 3.—Twenty-four female LOU (Louvain) rats, 2–6 months of age and weighing 100–200 g, were singly housed in plastic cages and standardized to a schedule of 12 hours of light alternating with 12 hours of darkness for 5 weeks before and throughout the study. On Nov 1, 1980, the rats were randomized to receive a single ip injection of cisplatin (5 mg/kg) or normal saline (controls) at 7 HALO or 19 HALO. These two circadian stages, respectively, correspond to the mean nadir and mean maximum of the circadian rhythm in urine volume of rats fed ad libitum (14). At 7 HALO 2.5 or 3 days later, the rats were weighed and rectal temperature was measured with a thermistor probe. The rats were then killed by decapitation and blood was collected. Serum was analyzed for BUN content. Both kidneys were removed, fixed in Formalin, and stained with hematoxylin and eosin.

Drug Preparation

Cisplatin was supplied by the Investigational Drug Branch, National Cancer Institute, in vials containing 10 mg of cisplatin plus mannitol and sodium chloride. The final solution used for injection contained 1 mg of cisplatin, 10 mg of mannitol, and 9 mg of sodium chloride per milliliter. Fresh drug was prepared for each injection.

Cisplatin Determination

Exactly 3.0 ml of urine was adjusted to pH 8 with 5% aqueous sodium carbonate solution, and 100 μl of 0.5 M aqueous diethylammonium diethyldithiocarbamate was added. The resulting solution was heated on the steam bath for 10 minutes and allowed to cool to room temperature, and exactly 3.0 ml of chloroform was added. This mixture was vortexed for 5 minutes, aqueous NaSH (1 ml, 0.2 M) was added, and vortexing was continued for an additional 2–3 minutes. The chloroform layer was removed and analyzed for platinum concentration by high-performance liquid chromatography as described previously (1). Each determination was carried out in duplicate on freshly thawed urine.

Previous studies have shown that most of the platinum excreted in the urine within 4 hours of iv administration is in the form of unchanged cisplatin (3). The amount of cisplatin in 1 ml of urine can be calculated by multiplying the concentration of platinum by the molecular weight of cisplatin divided by that of platinum, a factor of 1.54.

Blood Urea Nitrogen

BUN was determined in serum by the urease method.

Histopathologic Assessment

Hematoxylin-eosin-stained slides of a slice of one kidney per rat were examined with light microscopy by an observer who did not know the treatment group of the donor rat. A semiquantitative assessment of the number of naked nuclei present within the tubular lumen was tabulated as a measure of the severity of cisplatin-induced renal damage. A scoring system was devised which ranked the amount of tubular damage from 0 (very few nuclei within the tubular lumina) to 5 (many nuclei within the tubular lumina).

Statistical Methods

Student's t tests were used to compare treatment groups. In addition, the single and population mean cosinor methods (12,13,23) were employed to determine and quantify the characteristics of any observed circadian rhythm. A *P* value from an F test of the zero-amplitude hypothesis is obtained. The fit of a cosine function to the data yields three rhythm characteristics with their respective 95% confidence limits. These are the mesor (rhythm-adjusted mean), the double amplitude (total extent of *predictable* variation accounted for by the cosine curve best fitting the data), and the acrophase (timing of the maximum of this best-fitting cosine curve). A fourth value, the percent rhythm, is a measure of the overall variability of the data which is attributable to the rhythm present.

RESULTS

Body Weight Loss

Studies 1 and 2.—Rats kept in metabolic cages lost 16.4% ± 2.0% (mean ± SE) of their body weight within the 6-day span following cisplatin administration, as compared to 10.1% ± 0.6% for those animals housed in plastic cages (t = 7.4, $P < 0.001$). A difference between the two groups receiving cisplatin at different circadian stages was demonstrable (table 1), with statistical significance for the groups kept in plastic cages but not for the groups housed in metabolic cages.

Study 3.—All rats were weighed at 7 HALO approximately 3 days after cisplatin administration. The mean (± SE) body weight of control rats was 159.6 ± 2.7 g; rats receiving cisplatin 3 days earlier at 7 HALO weighed. 151.3 ± 3.3 g, while those receiving cisplatin 3 days earlier at 19 HALO weighed 160.5 ± 4.0 g (F = 3.2, $P = 0.09$).

TABLE 1.—Body weight loss of female F344 rats (kept in plastic cages) on Day 6 after a single ip injection of 5 mg/kg of cisplatin

	% body weight loss*	
Injection time	Mean ± SE	Significance
Daily urine volume maximum	9.6 ± 0.4	t = 2.15
Daily urine volume minimum	12.4 ± 1.3	$P < 0.05$

*Body weight of each rat measured at 8 HALO and expressed as a % of its preinjection value.

Urine Volume Increase

Urine production before and after cisplatin administration was evaluated in each individual rat. Cisplatin administration resulted in an overall increase in the 24-hour mean of urine volume. This increase was maximal near the fifth day following drug administration. The increase in urine excretion over the first 6 days following cisplatin administration depended markedly on the circadian timing of drug injection ($P = 0.025$) (table 2).

TABLE 2.—Cisplatin-induced polyuria in female F344 rats secondary to ip injection of 5 mg/kg of cisplatin*

	Integrated relative increase in urine volume	
Injection time	% × days (mean ± SE)	Significance
Daily urine volume maximum	593 ± 138	t = 2.9
Daily urine volume minimum	1396 ± 263	$P = 0.025$

*Area of relative response over 6 days following ip drug administration at circadian acrophase or bathyphase of urine volume.

Blood Urea Nitrogen

When cisplatin was given at 19 HALO, the BUN was only slightly elevated (25 ± 1 mg/dl) when compared with that of controls (17 ± 1 mg/dl), whereas the administration of cisplatin at 7 HALO resulted in a threefold rise in BUN (53 ± 5 mg/dl) (fig 1).

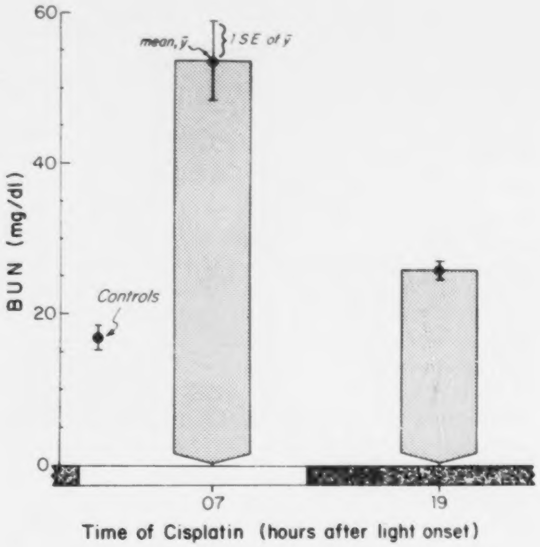

FIGURE 1.—BUN ~ 3 days after administration of single ip dose of cisplatin to female LOU rats on standardized schedules. Drug given at 7 or 19 HALO. Blood obtained at 7 HALO ~ 3 days later. Role of circadian time: t = 4.4, DF = 14, $P < 0.001$. Cisplatin at 19 HALO vs controls: t = 5.0, DF = 14, $P < 0.001$.

Histologic Changes

Histopathologic lesions predominated in the proximal tubule. They consisted of hydropic and necrotic changes. Proximal tubular cells appeared swollen. Caryorrhexis and pyknotic nuclei were noted. The scoring system revealed a mean ± SE of 0 ± 0 for controls, 2.9 ± 0.4 for rats receiving cisplatin at 7 HALO, and 1.7 ± 0.7 for rats receiving cisplatin at 19 HALO (t = 1.8, $0.10 < P < 0.20$). The correlation between histopathologic score and BUN was statistically significant (r = 0.70, $P < 0.05$).

Cisplatin Urinary Excretion

Figure 2 summarizes the urinary excretion of cisplatin. Irrespective of injection time, most cisplatin recovered in the urine was found in the first 8 hours following drug injection. Rats injected with cisplatin near the circadian maximum of urine volume excreted more of the drug in the Hour 4 urine sample and less drug in the Hour 8 sample than rats receiving cisplatin near the circadian minimum in urine volume.

The total urinary excretion of cisplatin (expressed as percent of the injected dose) in the first 20 hours following drug injection was 41.1 ± 3.6 in rats injected near the time of the daily maximum of urine volume and 33.2 ± 0.8 in rats injected near the daily nadir of urine volume (t = 2.0, $0.05 < P < 0.10$).

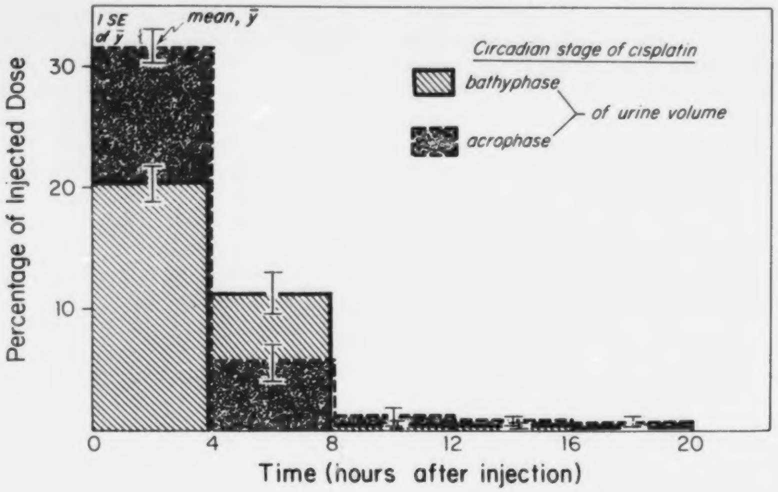

FIGURE 2.—Cisplatin urinary excretion expressed as % of injected dose recovered every 4 hrs over 20 hrs following ip administration of 5 mg/kg of the drug to female F344 rats on standardized schedules. Cisplatin was given at maximum or minimum of mean circadian rhythm in pretreatment urine volume.

Cisplatin Urinary Concentration

The urinary concentration of cisplatin in both studies is shown in figure 3. In the Hour 4 urine sample obtained from rats receiving cisplatin near the circadian maximum of urine volume, cisplatin concentration was one-half of that observed after cisplatin administration near the circadian minimum of urine volume ($P < 0.01$). This difference was even larger in the Hour 8 urine sample.

Table 3 demonstrates the effect of cisplatin timing on the area under the curve of urinary drug concentration during the first 20 hours following drug administration. Cisplatin urinary concentration may reflect the drug concentration to which proximal tubular cells are exposed. If so, during the 20-hour span following cisplatin injection, the tubular cells of rats receiving cisplatin near the time of day associated with the daily maximal urine volume were exposed to about half the cisplatin

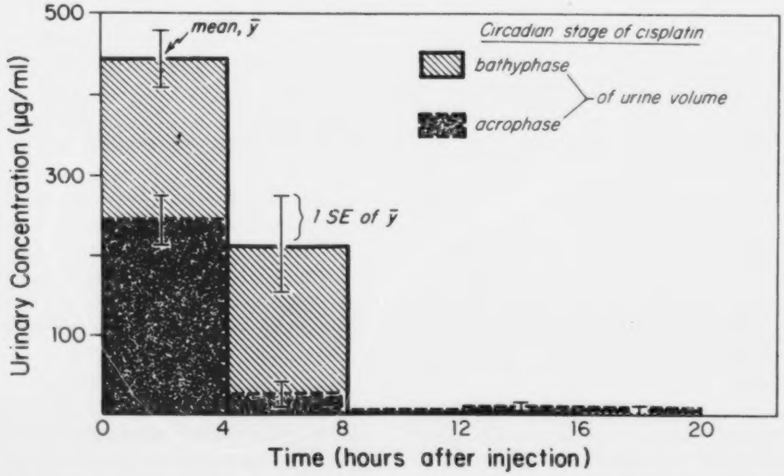

FIGURE 3.—Cisplatin urinary concentration of 4-hr urine specimens collected over 20 hrs following ip administration of 5 mg/kg of the drug to female F344 rats on standardized schedules. Cisplatin was given at maximum or minimum of mean circadian rhythm in pretreatment urine volume.

concentration as were the tubular cells of rats injected at the time of day associated with the daily minimum.

TABLE 3.—Total cisplatin urinary concentration over the 20-hr span (C × T) following ip administration of 5 mg/kg of the drug to F344 rats

	Total cisplatin concentration	
Injection time	C × T* (mean ± SE)	Significance
Daily urine volume maximum	1257 ± 197	t = 3.7
Daily urine volume minimum	2470 ± 278	$P = 0.01$

*μg × hr × ml^{-1}.

Urinary Pharmacokinetics and Nephrotoxicity of Cisplatin

A statistically significant correlation was demonstrated between the areas for cisplatin urinary concentration × time and for urinary volume response × time, evaluated over the first 6 days following drug injection (fig 4). A statistically significant correlation was also found between this apparent loss of urinary concentrating ability and the peak of cisplatin urinary concentration ($r = 0.79$, $N = 9$, $P < 0.025$).

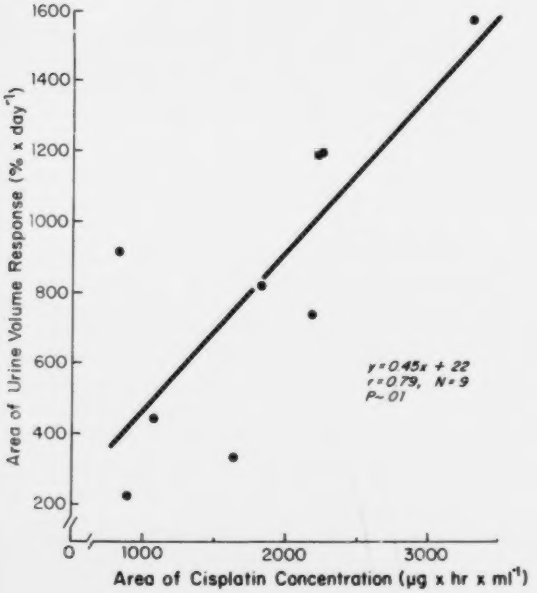

FIGURE 4.—Correlation between cisplatin nephrotoxicity gauged by urine volume response and urinary drug concentration in 9 female F344 rats singly housed in metabolic cages and standardized to light: darkness schedules of 8:16 hrs, with food availability restricted to first 2 hrs of darkness. Areas of cisplatin concentration over 20-hr span after cisplatin. Areas of relative urine volume response over 6-day span following drug injection.

DISCUSSION

Urinary volume increase as the result of a presumed loss of urinary concentrating ability has been demonstrated to be a sensitive index of cisplatin-induced renal damage in the rat (7,19). In these studies, we have found that this index of cisplatin nephrotoxicity depends largely on the circadian timing of a nonlethal dose of this drug. We also demonstrated that cisplatin-induced renal toxicity is circadian stage-dependent in two strains of rats under different standardized lighting and feeding regimens.

In study 3, the interval between cisplatin administration and blood sampling for BUN determination differed by 12 hours between animals injected at 7 or 19 HALO, because rats receiving cisplatin at circadian stages 12 hours apart were all sampled at the same time. Our choice of sampling all rats at the same circadian stage (7 HALO) as opposed to sampling all rats after the same span following cisplatin was essential to avoid the confounding effect of the normally occurring circadian rhythm in BUN. In both transverse and longitudinal study designs, we have shown that within a 24-hour range, the circadian stage of sampling is critical. This has proven to be true for assessing cisplatin renal toxicity by either systemic endpoints (body weight or temperature) or by the kidney-specific indicators of urine volume, BUN, or urinary *N*-acetylglucosaminidase activity (19,21).

According to Guarino et al (9), direct tubular exposure to a concentration of 200 μg/ml of cisplatin for a few hours is necessary for tubular damage to occur. Our data demonstrate that a concentration of this magnitude persists 6 ± 2 hours in the excreted urine if cisplatin is given at the time of day associated with the circadian minimum of urine volume, whereas it only lasts 2 ± 2 hours if the drug is given near the circadian maximum of urine volume.

The pharmacokinetics of cisplatin urinary concentration in the rat differ tremendously as a function of drug timing. The statistically significant correlation between these pharmacokinetic parameters and subsequent week-long loss of urinary concentrating ability supports the role of the urinary elimination of cisplatin in its nephrotoxicity. Although some authors infer a simple direct relationship between urinary peak concentration of cisplatin and the renal toxicity of this drug (2,9), our work suggests a similar but more complex hypothesis.

We have demonstrated elsewhere that cisplatin nephrotoxicity is correlated with the urinary activity of β-*N*-acetylglucosaminidase (NAG)—a lysosomal enzyme released into the urine from the brush border of proximal tubular cells. We have also reported that platinum uptake by many tissues is positively correlated with NAG tissue activity (20).

In the unperturbed state, a statistically highly significant circadian rhythm in urinary NAG is present. The circadian amplitude of this normal urinary rhythm approximates 100% of the 24-hour mean. Urinary NAG activity, as well as other urinary enzymatic activities specific to the proximal tubule, may reflect a highly specific index of the ability of platinum to enter the proximal tubular cell. Most likely, renal and subsequent urinary NAG activities reflect rhythmic renal metabolic activity. Proximal tubular reabsorption is maximal when the activities of NAG and other enzymes are highest. Cisplatin uptake by these proximal tubular cells and the resulting cellular damage may depend on the *interaction* of (*a*) the circadian stage of metabolic activity of the renal tubular cells as reflected by their NAG content at the time of cisplatin exposure and (*b*) the urinary concentration of cisplatin in the proximal tubule. The concentration of cisplatin in the excreted urine depends on both of these parameters.

This hypothesis can help explain why the degree of renal protection which results from the concurrent administration of identical hydration regimens is either trivial or very large, depending on whether cisplatin is given at a circadian stage of maximal or minimal metabolic activity of the proximal tubular cells, respectively (17,20).

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Toxicology of AMSA in Beagle Dogs and CDF_1 Mice[1,2]

Kenneth L. Pavkov[3,*] **and Daryl C. Thake**[4,5]

The toxic effects of AMSA in single-dose (× 1) or five-daily-dose (× 5) regimens were studied in male and female beagle dogs and CDF_1 mice. AMSA was administered orally via gelatin capsules to dogs and as a Klucel suspension to mice. Suitable placebo controls were evaluated. Doses in dogs ranged from 62.5 to 1000 mg/m^2 (lethal dose [LD]) for the × 1 study and from 31.25 to 500 mg/m^2 (LD) for the × 5 study. Dogs given the LD (× 1 and × 5) had degenerative lesions in the gastrointestinal mucosa, depletion of bone marrow hematopoietic tissue, and lymphoid depletion. These lesions were not seen at doses lower than the LD in the × 1 study but were present in the × 5 study, with severity related to dose. Toxicity appeared to be reversible at the lower doses since animals killed after a 45-day observation period had none of the above lesions. Clinical signs of emesis, diarrhea, and weight loss correlated with the above lesions as did the depressed wbc counts. The toxicity in dogs was dose- and schedule-dependent. The 14-day LD10, LD50, and LD90 values (mg/m^2) for mice in the × 1 study were as follows: LD10, 440 in males and 475 in females; LD50, 810 in males and 728 in females; and LD90, 1489 in males and 1117 in females. Mortality was observed initially on Day 4. Single-dose toxicity studies were conducted in mice by oral administration of doses equal to one-half of the LD10, the LD10, and the LD50. The major drug-related lesions in mice included thymic degeneration and atrophy and bone marrow depletion at the higher dose levels, while the major drug-related lesions in dogs included enteric mucosal degeneration and generalized lymphoid and bone marrow depletion. The toxicity of AMSA was reversible in mice and dogs. [Cancer Treat Rep 66:1939–1943, 1982]

The Investigational Drug Branch of the National Cancer Institute (NCI) is presently sponsoring both phase I and II clinical evaluations of AMSA, an acridine derivative (1,2). The promising efficacy of AMSA in the murine tumor models, reported by Cain and Atwell (1) and reviewed by Von Hoff et al (3), has promoted keen interest in this new class of antineoplastic drugs. In preclinical studies of AMSA using an iv formulation, it was found that a solution containing 5 mg/ml of AMSA, 2.8 mg/ml of L-lactic acid, and 100 mg/ml of anhydrous *N,N*-dimethylacetamide (DMA) (pH 3.5) or the vehicle alone (equivalent amounts of DMA and L-lactic acid) (pH 3.2) caused overt soreness and local tissue inflammation in guinea pigs and rabbits. The investigators thought the low pH of the formulation was responsible for the irritation (4). Iv infusion of AMSA, formulated as above, in the phase I (3,5,6) and II (7) clinical evaluations has resulted in phlebitis, a major discomforting adverse effect.

Cysyk et al (8) showed that route of administration of AMSA affects the plasma clearance and tissue distribution and that an oral formulation might be desirable. Further, Cysyk suggested that rapid hepatic clearance and biliary excretion of orally administered AMSA might reduce distribution to sensitive tissues and protect against the dose-limiting cytotoxicity associated with iv dosing. Further, orally absorbed AMSA might have target effectiveness against primary or metastasized tumors in the liver.

This preclinical investigation was undertaken to evaluate the toxicity of AMSA administered orally to beagle dogs and CDF_1 mice.

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[1] Received Dec 9, 1981; revised June 8, 1982; accepted July 13, 1982.

[2] Supported by contract N01-CM-43746 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The full report is available through the National Technical Information Service, NTIS-No. BCL-KPDT-249992-79-2.

[3] Battelle, Columbus Laboratories, OH.

[4] Monsanto, St. Louis, MO.

[5] We thank Mr. Chris Rahrig and Ms. Barbara Wood for their skillful technical assistance and Dr. Steven Weisbrode, The Ohio State University, for his assistance in pathology.

[*] *Reprint requests to:* Dr. Kenneth L. Pavkov, Toxicology and Health Sciences Section, Battelle, Columbus Laboratories, 505 King Ave, Columbus, OH 43201.

MATERIALS AND METHODS

AMSA

AMSA, supplied as bulk powder (lot No. PH41-75-1, sample NA) was obtained from Starks Associates, Inc, Buffalo, NY, through the NCI. The chemical identity of AMSA was verified by infrared spectroscopy, nuclear magnetic resonance, and melting point (228°C–231°C) determination. The purity was estimated to be $> 95\%$ since no unidentifiable signals were observed in the nuclear magnetic resonance spectra. The oral dose was prepared fresh daily in gelatin capsules for dogs or suspended (10 mg/ml) in Klucel,[6] with continuous stirring, for oral gavage administration to mice.

Animal Studies

The protocols were from the procedures outlined by Prieur et al (9) and included a toxicity study in mice as described later.

Purebred beagle dogs, 6–7 months of age with mean body weights of 11.7 kg (range, 9.4–13.4) for males and 9.0 kg (range, 7.8–10.0) for females, were supplied by Marshall Research Animals, Inc, North Rose, NY, through the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment (DCT), NCI. The dogs, certified by the breeder to be free of heartworms and immunized against distemper, hepatitis, and leptospirosis, were given a complete physical examination upon receipt and quarantined for a minimum of 2 weeks. These animals were housed individually in stainless-steel cages, fed 400 g of Purina High Protein Dog Chow once each day, and provided water ad libitum. All dogs were identified by a unique permanent numerical tattoo, and the cages were appropriately marked with this number, a study identification, the date of initial treatment, and dose regimen. Fecal examinations for parasites, a minimum of two complete hematologic and serum chemistry profiles, one urinalysis examination, and an ophthalmoscopic examination were performed for each dog prior to initiation of the study. Body weight and rectal temperature were determined a minimum of once per week. After treatment with AMSA, the parameters that were analyzed included hematologic and serum chemistry determinations and urinalyses on Days 2, 4, and 8, and weekly thereafter. Examinations for clinical signs of toxicity were performed daily and final ophthalmoscopic and complete physical examinations were followed by necropsies and histopathologic evaluations. The dogs were treated in pairs, one male and one female at each dose level, according to the treatment schedule shown in table 1.

TABLE 1.—Treatment schedules and dose ranges used for toxicologic evaluation of AMSA administered orally in beagle dogs and CDF_1 mice

Species	Treatment schedule	Dose range (mg/m^2)*
Dogs†	×1	62.5–1000.0
	×5	31.25–500.0
Mice‡	×1	220.5–809.7 (males) 237.0–728.4 (females)

*Doses in mg/m^2 were estimated by multiplying the mg/kg doses by a factor of 20 for dogs and 3 for mice (ref 10).

†Dose levels for dogs were either doubled or reduced by $^1/_2$ until a lethal dose and a nontoxic dose were established. Toxicity levels (ref 9) for dogs in this study were defined as follows. *Highest nontoxic dose (HNTD)*—the highest dose at which no hematologic, chemical, clinical, or morphologic drug-induced alterations occur; doubling this dose should produce these alterations. *Toxic dose low (TDL)*—the lowest dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose should not produce lethality. *Toxic dose high (TDH)*—the dose which produces drug-induced pathologic alterations in hematologic, chemical, clinical, or morphologic parameters; doubling this dose produces lethality. *Lethal dose (LD)*—the lowest dose which produces drug-induced death during the treatment or observation period.

‡Dose levels for mice were $^1/_2$ the defined LD10, the LD10, and the LD50.

In the single-dose (×1) study in dogs, six pairs were used. Five pairs were treated orally with single doses of 50, 25, 12.5, 6.25, or 3.125 mg/kg (1000, 500, 250, 125, or 62.5 mg/m^2) to obtain the defined toxicity levels (table 2). One pair of dogs was used as a study control and was given a number of empty gelatin capsules equal to the number used to deliver the largest dose. In the repeated-dose (×5) study in dogs, six pairs were used. Five pairs were treated orally with five consecutive daily doses of 25, 12.5, 6.25, 3.125, or 1.562 mg/kg (500, 250, 125, 62.5, or 31.25 mg/m^2) and one pair was given empty gelatin capsules and served as a study control. The most severely affected animal of each dose pair was killed by overdosing with sodium pentobarbital on Day 8 for the ×1 studies and on Day 6 for the ×5 studies. The remaining partner was kept for long-term observation and was killed on Day 46 or Day 52 for the single- or multiple-treatment studies, respectively. All tissues were fixed in 10% neutral buffered Formalin and sections were stained with hematoxylin and eosin for later evaluation. The pituitary, adrenal glands, thyroid, brain, testes or ovaries, liver, kidneys, and heart (left and right ventricles, interventricular septum and atria) of each dog were weighed and ratios of organ to final body weight were computed.

Male and female CDF_1 mice were supplied by Harlan Industries, Inc, and Laboratory Supply Co, Inc, Indianapolis, through the Mammalian Genetics and Animal

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[6] 0.3% Hydroxypropyl cellulose. Screening Program, Drug Evaluation Branch, NCI.

TABLE 2.—Defined toxicity levels of AMSA administered orally in beagle dogs and CDF$_1$ mice

Species (sex)	Study	Toxicity*	Dose	
			mg/kg	mg/m^2
Dog (M,F)	×1	LD	50.0	1000.0
		TDH	25.0	500.0
		TDL	6.25	125.0
		HNTD	3.125	62.5
Dog (M,F)	×5	LD	25.0	500.0
		TDH	12.5	250.0
		TDL	3.125	62.5
		HNTD	1.562	31.25
Mouse (M) 14-day lethality		LD90	496.2	1488.6
		LD50	269.9	809.7
		LD10	146.8	440.4
Mouse (F) 14-day lethality		LD90	372.4	1117.2
		LD50	242.8	728.4
		LD10	158.3	474.9

*See table 1 for definitions.

Production Section, DCT, NCI. Mice were quarantined for a minimum of 1 week prior to test. Mice were housed in polycarbonate cages with chipped hardwood bedding. Purina Rodent Chow 5001 and water were available ad libitum except during the 6-hour predosing fast when only food was withheld. In the lethality study, single oral gavage doses were administered to ten animals of each sex (21–24 g of body weight) and the mice were observed for 14 days. One-half of the determined LD10, the LD10, and the LD50 doses and Klucel (vehicle) and untreated control groups were used to determine drug toxicity in six mice per sex per dose. All animals treated with the LD50 dose were killed on Day 3 (Day 1 was the day of treatment) along with one-half of the mice in each of the other study groups. The remaining animals were killed on Day 15 (14th observation day). The toxicity end points included clinical signs and body weights during the observation period and hematologic and selected serum chemistry analyses (BUN, SGOT, and SGPT) on the termination day. Necropsies and histopathologic evaluations were performed on each animal.

RESULTS

Single-Dose Study in Dogs

Clinical response.—The male treated with 50 mg/kg died on Day 5, whereas the female, after showing the toxic signs described below, returned to a clinically normal condition and survived until the scheduled termination on the 46th test day. Within 3 hours after treatment, both animals at the highest dose had a 3°F drop in rectal temperature, and the female had mucoid (orange-colored) stool. In the male, emesis, depression, anorexia, diarrhea, and a 19% loss of initial weight were observed during the first 5 days. The female had mucoid diarrhea on Days 3, 30, and 31 but did not show other signs of toxicity. Mucoid diarrhea, soft stool, emesis, and slight weight loss were observed in dogs from nonlethal dose groups, but these effects did not appear to be dose-related. Leukopenia was dose-related at all the toxic dose levels with nadirs during the first week after treatment. Dogs kept for extended observation quickly returned to normal. Reticulocytopenia was observed during the first week after treatment in dogs treated at the 50-, 25-, and 12.5-mg/kg dose levels and returned to normal in the second week. Although this response was extensive at the two highest doses, the number of circulating erythrocytes was not dramatically affected, and nucleated rbcs were never observed in the peripheral blood samples.

Pathology.—Tissue changes that were attributed to a toxic drug effect occurred mainly in the one dog that died on Day 5 following treatment with 50 mg/kg. In the small intestine the lesions were characterized by mucosal degeneration with crypt cell changes indicative of cell cycle alterations. Congestion in the mucosa was present in the ileum and colon, and hemorrhage occurred in the jejunal mucous membrane. Degenerative changes were also apparent in the glandular epithelium of the esophagus in this animal. A generalized lymphoid depletion of moderate to severe intensity involved all lymphoid organs examined (spleen, lymph nodes, thymus, and Peyer's patches). The hematopoietic elements of the bone marrow were also severely depleted. Centrilobular regions of the liver were markedly congested with mild necrosis. Other lesions included moderate hemorrhage in the mesenteric lymph node and moderate vacuolation of the cells of the zona glomerulosa and zona fasciculata of the adrenal cortex. The only drug-related lesions in the high-dose animal held for extended observation occurred in the liver and included diffuse vacuolation of centrilobular hepatocytes and dilation of the portal lymphatic vessels. Other treatment-related lesions were observed in dogs from the intermediate dose level: 12.5 mg/kg. The female killed on Day 8 had mild but generalized erythrophagocytosis and hemorrhage in the lymph nodes and the male killed on Day 46 had mild centrilobular hepatic necrosis. There were no drug-related lesions observed in dogs treated with 25, 6.25, or 3.125 mg/kg of AMSA. Organ weights (absolute and relative to final body weight) were within normal limits.

Five Daily Doses in Dogs

Clinical response.—The highest dose level, 25 mg/kg, was lethal to the female on Day 6 and to the male on Day 7. Toxic effects observed in this treatment regimen were very similar to those seen in the single-dose treatment. Depression and lethargy were observed toward the end of the treatment period in both dogs given the 25-mg/kg dose. Diarrhea and emesis were seen on the third through fifth treatment days (6–24 hours after treatment) at the two highest dose levels. Body weights decreased by 12%–13% on Day 6 in the lethal-dose dogs and by 7%–9% in dogs at the 12.5-mg/kg dose. The body weight of the male held for extended observation (47-day recovery period) returned to near normal by Day 52. The manifestations of clinical signs at the lower doses were not consistently dose-related. The female treated with 6.25 mg/kg daily for 5 days had mucoid diarrhea on treatment Days 3, 4, and 5 and diarrhea on Day 6. Although there were no clinical signs of drug toxicity such as emesis or diarrhea at the 3.125-dose level, the female treated with 1.562 mg/kg/day for 5 days had emesis on Days 1 and 3 and diarrhea on Days 3 and 5. Decreases in reticulocyte and leukocyte populations were observed at all dose levels. Severity, time of onset, and duration of response for these parameters were directly related to dose level. Rapid reversibility of these effects was seen in all animals held for the extended observation period. At the 25-mg/kg dose level there was evidence of hemoconcentration (hematocrit of 54%) in the male prior to death. There were slightly elevated BUN values (increases to 25 and 23 mg/100 ml in the male and female, respectively) just prior to death, and the SGOT was increased to 33 IU in the female on the second day of treatment. At the lower dose levels, neutropenia and lymphopenia were observed. Decreases in circulating myeloid and erythroid elements occurred in conjunction with depleted bone marrow in the female at the 12.5-mg/kg dose level. The male from this dose group had decreased hematocrit and hemoglobin values and decreased reticulocyte, erythrocyte, and leukocyte counts during and immediately following the 5-day treatment period. In a dose-related fashion, these parameters were depressed and returned to normal during the 47-day observation period, indicating the reversibility of the bone marrow suppression. Serum chemistry values fluctuated mildly at the lower dose levels but did not indicate toxic effects.

Pathology.—The dogs that received 25 mg/kg/day for 5 days died on Days 6 and 7 and had lesions similar to those described for the male dog treated with a single 50-mg/kg dose of AMSA. Mucosal degeneration of the small intestine and colon, moderate to severe lymphoid depletion or necrosis in most lymphoid tissues, and severe bone marrow depletion were present. At the lower dose levels, the severity of these lesions was related to dose level and occurred in the animals killed on the day following the fifth and last treatment. No drug-related pathologic lesions were observed in animals killed following the 47-day observation period. Organ weights were within normal limits.

Median Lethal Dose in Mice

For CDF_1 mice, the 14-day, oral LD50 was found to be 269.9 mg/kg (809.7 mg/m^2) for males and 242.8 mg/kg (728.4 mg/m^2) for females. The 95% confidence intervals were 237.4–306.8 mg/kg and 213.2–276.4 mg/kg, respectively. The LD10 was 146.8 mg/kg (440.4 mg/m^2) for males and 158.3 mg/kg (474.9 mg/m^2) for females, with 95% confidence intervals of 100.8–213.7 mg/kg and 118.0–212.2 mg/kg, respectively. The median lethal doses were calculated for combined sexes for comparison with previously published data and are shown in table 3. Mortality occurred from Day 4 through Day 8 in males and from Day 4 through Day 9 in females. For both sexes the majority of deaths at each dose level occurred from Day 4 through Day 6. The modal-day of death for each sex was Day 5. Piloerection and lethargy were the dose-related clinical signs of toxicity through Day 10.

Single-Dose Acute Toxicity in Mice

All animals treated at the defined male and female LD50 dose levels were killed on the third day of the study with no apparent clinical abnormalities. Treatments at the LD50, the LD10, and one-half the LD10 suppressed the hematopoietic tissues of mice as shown in the abnormal hematologic parameters in animals killed on test Days 3 and 15. Toxic signs at the lower doses included weight loss, piloerection, and lethargy from Days 4 through 10. The male mice given the LD10 dose and not killed on Day 3 all died on Day 8. One female at the LD10 dose level died on Day 6 and had severe degeneration and necrosis of the thymus and severe atrophy of the bone marrow. The histopathologic eval-

TABLE 3.—Comparative lethal single-dose levels of oral and iv AMSA in CDF_1 mice

	Defined toxicity levels (mg/m^2)*		
Route	LD90	LD50	LD10
Oral	1270.2 (1105.8–1458.9)	761.4 (704.7–822.9)	456.6 (375.3–555.3)
Iv†	111.9 (107.7–116.4)	101.1 (98.7–103.5)	91.2 (87.3–95.7)

*Values in parentheses = 95% confidence intervals.

†Ref 4 and Henry et al (Oct 20, 1976) Report No. IITRI-Tox-249992-76-2 to DHEW-PHS, NIH-NCI, Laboratory of Toxicology, Bethesda, MD 20205.

TABLE 4.—Comparative toxic dose levels of oral and iv AMSA in beagle dogs

		Defined toxicity levels (mg/m^2)			
Study	Route	LD	TDH	TDL	HNTD
×1	Oral	1000.0	500.0	125.0	62.5
	Iv*	125.00	62.60	31.24	15.62
×5	Oral	500.0	250.0	62.5	31.25
	Iv*	15.60	7.80	3.90	1.96

*Ref 4.

uations of mice killed on Day 3 showed mild to marked lymphoid depletion of the cortical thymus and mild to severe atrophy of the bone marrow. These lesions were not seen in mice given the LD10 (female) or one-half of the LD10 (male or female) and killed on Day 15.

DISCUSSION

Our studies indicated that the oral administration of AMSA induced toxic changes to bone marrow, lymphoid tissue, intestinal mucosa, and liver. These toxic effects were similar to those reported by Henry et al (4) for iv administered AMSA. However, we did not observe CNS or renal toxic effects either by clinical or pathologic evaluations. This might be expected since earlier studies by Cysyk et al (8) showed that the oral route of administration resulted in considerably lower levels of unchanged AMSA in kidney and brain tissues compared to levels found following iv administration. As shown in tables 3 and 4, using the same definition of toxicity, the tolerable dose levels in our study are higher than those reported earlier for iv administration. It may be possible to direct higher doses of AMSA to specific tumor sites in clinical studies using the oral route of administration. This approach would avoid iv infusion and the associated phlebitis.

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Activity of 1-*p*-(3,3-Dimethyl-1-triazeno) Benzoic Acid Potassium Salt in M 5076/73A Ovarian Reticular Cell Sarcoma of the Mouse[1,2]

T. Colombo,[3] S. Garattini,[3] L. Lassiani,[4] and M. D'Incalci[3,*]

1-*p*-(3,3-dimethyl-1-triazeno) benzoic acid potassium salt (DM-COOK) was tested on M 5076/73A (M5) mouse sarcoma at a dose of 40 or 50 mg/kg/day (Days 6–14) after im transplant of 7 × 10^5 cells, with or without surgical removal of the primary tumor on Day 14. Treatment at either dose level resulted in reduction of the primary tumor weight to around 50% of that in the controls, and striking antimetastatic effects were observed. When a dose of 40 or 50 mg/kg of DM-COOK was followed by surgery, there were 14% and 40% long-term survivors, respectively, but the higher dose caused about 30% toxic deaths. After iv injection of 10^3 or 10^5 M5 tumor cells, no artificial metastases appeared in DM-COOK-treated mice, whereas all control animals had metastatic involvement in the liver, spleen, ovaries, and kidneys. [Cancer Treat Rep 66:1945–1948, 1982].

Aryl-dimethyltriazenes (DMTs) are antitumor agents that have shown activity in a number of experimental tumors in the mouse (1,2). They were initially developed with the idea that dacarbazine, which is clinically used for treatment of melanoma and soft tissue sarcoma (3–5), could be replaced by other DMTs with greater stability and forming fewer diazonium ions, which are considered to be responsible for toxicity but not for antitumor activity (6).

The mechanism of action of DMTs is still unknown, but one possibility is that it is mediated by the monomethyltriazenes, potent alkylating agents formed by oxidative *N*-demethylation of the parent compound (7). Some other bioproducts of DMTs, however, may have more selective action against tumors sensitive to these compounds (8). In addition it has been suggested that some DMTs exert an antimetastatic effect independently of their cytotoxic effect. Sava et al (9) reported that 1-*p*-carboxamidophenyl-3,3-dimethyltriazene and 1-*p*-carboxyphenyl-3,3-dimethyltriazene and its potassium salt DM-COOK displayed a selective antimetastatic effect on Lewis lung carcinoma; no lung metastases were found after DM-COOK treatment. Conversely, when these tumor cells were injected iv, DM-COOK had no effect on the artificial metastases growing in the lung, suggesting that the antimetastatic effect was not directly related to its cytotoxic properties (9,10). The authors therefore speculated that the compound might act by preventing metastatic dissemination.

In this study in the mouse we investigated the antitumor and antimetastatic effects of DM-COOK, with and without surgery of the primary tumor, on the ovarian reticular cell sarcoma M 5076/73A (M5), which metastasizes preferentially to peritoneal organs (11,12).

MATERIALS AND METHODS

Animals and Tumor

Female C57BL/6J mice (20 ± 2 g) obtained from Charles River Laboratories, Italy, received an im transplant of 7 × 10^5 M5 cells. The M5 ovarian sarcoma was supplied by Mason Research Institute, Division of Cancer Treatment, Animals and Human Tumor Bank, Worcester, MA. Artificial metastases were obtained by iv injection of 10^3 or 10^5 tumor cells.

Treatment

The animals were injected ip with 0.1 ml/10 g of the drug freshly prepared in the dark as a solution in 0.1 M $NaHCO_3$. Treatment was given daily for 9 consecu-

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[1] Received Dec 29, 1981; revised July 8, 1982; accepted Aug 5, 1982.

[2] Supported in part by Public Health Service grant CA-12764 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and CNR contract No. 80.01539.96. The generous contribution of the Italian Association for Cancer Research is gratefully acknowledged.

[3] Istituto di Ricerche Farmacologiche "Mario Negri," Milan, Italy.

[4] Istituto di Chimica Farmaceutica, Università di Trieste, Italy.

**Reprint requests to:* M. D'Incalci, MD, Istituto di Ricerche Farmacologiche "Mario Negri," Via Eritrea 62, 20157 Milan, Italy.

TABLE 1.—Antitumor and antimetastatic activity of DM-COOK (ip), with or without surgery, in mice bearing M5 ovarian sarcoma[*]

Dose (mg/kg)	Surgery	Primary tumor weight at surgery in g (mean ± SE)	Toxic deaths (%)	Animals with metastases at death (%)			T/C (%)[†]	Long-term survivors (%)[‡]
				Liver	Ovaries	Spleen		
—	No	—	—	100	18	0	—	0
40	No	—	0	18	0	0	148	0
—	Yes	1.75 ± 0.10	—	100	40	7	—	0
40	Yes	0.70 ± 0.03[§]	0	7	0	0	250	14
—	No	—	—	100	7	0	—	0
50	No	—	33	20	7	0	100	0
—	Yes	1.44 ± 0.05	—	100	27	7	—	0
50	Yes	0.68 ± 0.02[§]	27	0	0	0	132	40

[*] Each group consisted of 15 mice. Treatment was given daily for 9 consecutive days, starting on Day 6 after tumor transplantation. Surgery was carried out on Day 14 after tumor transplantation.

[†] T/C (%) = median survival time of treated mice/median survival time of untreated controls, × 100.

[‡] % treated animals surviving > 150 days after tumor implantation.

[§] $P < 0.01$ vs respective controls (Student's t test).

tive days, starting on Day 6 after tumor implantation. Control animals received the vehicle only.

Surgical Tumor Removal

The tumor was removed surgically 14 days after transplantation. Animals were anesthetized with ether, an incision was made above the tumor, the femoral artery and adjacent blood vessels were tied with sterile surgical silk thread, and the tumor-bearing leg was removed. The wound was washed with sterile normal saline and closed with Michell 7-mm wound clips. No mortality, no primary tumor recurrence, and no bacterial infections were observed after surgery.

Evaluation of Effects

In one experiment, autopsy was performed on Day 37 after transplant; in the others, autopsy was performed within 12 hours of death. The tumor weight was assessed by weighing the entire leg with the tumor. The weight of the tumor was not considered in experiments without surgical treatment, because cannibalism made it impossible to assess it with sufficient accuracy. Metastases were assessed by weighing the organs with metastases and by gross inspection. The median survival time was calculated as described by Geran et al (13). Death was classified as toxic death when it occurred before the first death among untreated control animals.

RESULTS

Table 1 reports the effect in mice bearing M5 ovarian reticular cell sarcoma of DM-COOK at daily doses of 40 or 50 mg/kg for 9 days starting on Day 6 after tumor transplantation, with or without surgery. At both dose levels DM-COOK showed a striking antimetastatic effect, causing reduction of the primary tumor and prolonging survival time, particularly when the treatment was followed by surgical eradication of the primary tumor. The 50-mg/kg dose appeared to be very active, inducing 40% cures in the mice from which the tumor was removed, but approximately 30% toxic deaths were observed. The 40-mg/kg dose was much better tolerated and showed similar activity on primary and secondary

TABLE 2.—Antitumor and antimetastatic activity of DM-COOK (ip), with surgery, in mice bearing M5 ovarian sarcoma[*]

Dose (mg/kg)	Weight (mean ± SE)			Animals with metastases (%)		
	Liver (g)	Ovaries (mg)	Spleen (mg)	Liver	Ovaries	Spleen
—	1.37 ± 0.297	67.9 ± 21.6	154 ± 13	54	100	0
40	0.646 ± 0.006	9.2 ± 1.3[†]	94 ± 12[‡]	0	0	0

[*] Each group consisted of 10 mice. Treatment was given daily for 9 consecutive days, starting on Day 6 after tumor transplantation. Surgery was carried out on Day 14 after tumor implantation (tumor weight at this time was 1.10 ± 0.05 g for controls and 0.61 ± 0.02[‡] g for treated animals). Antimetastatic activity was evaluated 37 days after tumor transplantation.

[†] $P < 0.05$ vs controls.

[‡] $P < 0.01$ vs controls.

Table 3.—Effect of DM-COOK on artificial metastases*

No. of tumor cells injected iv/mouse	Dose (mg/kg, ip)	Weight (mean ± SE)				Animals with metastases (%)			
		Liver (g)	Ovaries (mg)	Spleen (mg)	Kidneys (mg)	Liver	Ovaries	Spleen	Kidneys
10^3	—	1.708 ± 0.145	17 ± 3	259 ± 22	206 ± 9	100	7	93	14
10^3	40	0.966 ± 0.044†	14 ± 1	184 ± 13†	203 ± 7	0	0	0	0
10^5	—	1.886 ± 0.147	27 ± 3	265 ± 21	202 ± 7	100	71	100	93
10^5	40	1.078 ± 0.062†	12 ± 1†	162 ± 12†	210 ± 5	0	0	0	0

*Each group consisted of 14 mice. Animals were killed and examined for metastases on Day 18 from tumor cell injection.

†$P < 0.01$ vs respective controls (Student's t test).

tumors. A considerable number of late deaths from undetermined cause (81–118 days after tumor implantation) were observed among animals treated with DM-COOK and surgery. We noted only a weight loss (6–8 g) in the animals before death and found no macroscopic neoplastic lesions at autopsy.

Table 2 shows the results of DM-COOK treatment with doses of 40/mg/kg for 9 days before surgery, assessed by autopsy on Day 37 after tumor transplantation. This experiment confirms the clear-cut antimetastatic effect of DM-COOK in M5-bearing mice, as all the animals were apparently free of metastatic involvement in liver and ovaries, whereas these organs had metastases in most control animals. The drug also induced regression of the primary malignancy, as assessed by weighing the tumor removed at surgery. To shed some light on the antimetastatic mechanism of DM-COOK we evaluated the drug's effect on artificial metastases obtained by iv injection of 10^3 or 10^5 cells. As shown in table 3, no metastases appeared in DM-COOK-treated animals, whereas all animals of both control groups had metastatic involvement of the liver, spleen, ovaries, and the kidneys.

The unexpectedly large weight of the spleen in the experiments of treated animals without macroscopic metastases, contrasting with the finding of the previous experiment (table 2), prompted us to investigate whether DM-COOK treatment influenced the size of the organs evaluated in this study. We found that in non-tumor-bearing mice, spleen weight was significantly increased 18 days after the beginning of DM-COOK treatment with 9 daily doses of 40 mg/kg (60 ± 2 mg for controls vs 92 ± 5 mg for treated animals; $P < 0.01$). However, 37 days after the start of treatment, spleen weights were normal again (71 ± 2 mg for controls vs 72 ± 2 mg for treated animals).

The weights of liver, kidney, intestine, and ovary were unchanged 18 and 37 days after the start of treatment.

DISCUSSION

DM-COOK appears to be an active antitumor agent against mouse M5 reticular cell sarcoma of the ovary, showing a more marked effect on metastatic lesions than on the primary tumor. This was clear in all of the studies described, but when treatment was followed by surgery, a conspicuous increase in survival time was observed. These data are consistent with previous reports of the antimetastatic properties of this compound in another murine transplantable tumor, Lewis lung carcinoma (3LL), which results in metastatic deposits in the lung (9,10).

Metastases have been observed to have greater sensitivity than primary tumor in studies of several other drugs; this may be related to the faster kinetic rate of metastatic cells (14–15) and to the greater availability of the drug or its active metabolites (16).

Giraldi et al (10) claim that, in 3LL, DM-COOK has selective antimetastatic properties likely to be related to the inhibition of dissemination mechanisms. In our experimental conditions, however, DM-COOK showed a definite cytotoxic activity against M5 tumor cells. This is consistent with the reported cytotoxic activity of other phenyl-dimethyltriazenes (6) and is likely to be related to the formation of reactive metabolites; monomethyl derivatives, for example, are potent methylating agents (6,7).

The difference between our findings and Giraldi's may be due to the lower sensitivity of 3LL cells to the cytotoxic action of DM-COOK; they may become susceptible only when a relatively low number of cells disseminate into the bloodstream, becoming directly exposed to the drug or its active metabolites. This explanation is also suggested by previous experiments in this laboratory in which iv injection of 2 × 10^5 3LL cells was followed by a scant inhibitory effect on the formation of lung metastases, but a 50% reduction was evident after injection of 5 × 10^4 cells.

DM-COOK as a cytotoxic compound appears to be worthy of further investigation in other experimental tumors because it has striking antitumor activity, at least in the M5 tumor. The observed spleen enlargement caused by DM-COOK and the late deaths without evidence of disease are two points that also require further study.

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Hematopoietic Stem Cells in Friend Murine Leukemia Virus-Infected Mice Undergoing Chemotherapy: Effects of 5-FU[1,2]

H. J. Seidel* and Ludwika Kreja[3,4]

Uninfected DBA/2 mice and those infected 12 days previously with the polycythemia-inducing Friend murine leukemia virus were treated with 5-FU in doses of 18.75–200 mg/kg ip, and the effect on spleen weight, reticulocytosis, and hematopoietic stem cell compartments CFU-S, CFU-C, and CFU-E was studied. The CFU-E population of virus-infected mice, which had been completely independent of erythropoietin (Ep) in vitro before treatment, showed a considerable proportion of normal Ep-requiring CFU-E at Days 2–6 after doses ≤ 50 mg/kg, indicating a chemotherapy-induced remission. Later, Ep-independent growth was seen again, most probably due to a reinfection by the virus at the target cell level. The regeneration of all stem cell types was quicker in uninfected mice than in those infected with virus. These data are discussed in relation to results obtained with hydroxyurea treatment, where Ep-dependent CFU-E were also seen, and with respect to the effects of busulfan, carmustine, and dactinomycin, with their different modes of action. The importance of effective antiviral therapy for the cure of such a virus-induced malignancy is emphasized. [Cancer Treat Rep 66:1949–1956, 1982]

The chemotherapy of leukemia has two goals that are both indispensable for a complete remission and cure from the disease: elimination of the leukemic cell population and re-establishment of a normally functioning hematopoietic cell system.

The study of these parameters is facilitated if the leukemic cells can be quantified, eg, by use of a marker. In the past we have shown in the experimental Friend murine leukemia (polycythemia) model that the apparent erythropoietin (Ep)-independent growth of the Friend virus-transformed erythroid cell system can be used as such a marker; only transformed erythroid-committed precursors (CFU-E) form colonies in vitro without addition of Ep (1–5). In a study using multiple doses of hydroxyurea, we were able to kill all Ep-independent CFU-E; during the regeneration of the hematopoietic cell system, which started at a pluripotent stem cell (CFU-S) level that was 20% of that of controls, a wave of normal Ep-dependent CFU-E was seen, indicating the reinduction of normal hematopoiesis (6).

This finding was interpreted in accordance with the idea of a structuredness of the stem cell pools as proposed by Rosendaal et al (7): that previously resting and non-virus-infected stem cells survived the treatment and gave rise to this normal, Ep-dependent erythropoiesis in which, however, cells were reinfected by the virus after some days. In the studies presented in this paper we used 5-FU. This drug inhibits thymidylate synthetase and thus DNA synthesis, but in contrast to hydroxyurea, its killing effect is not restricted to cells in the S-phase (8). Rapidly proliferating stem cells like CFU-E and colony-forming units in culture (CFU-C) are more sensitive than CFU-S; a review of 5-FU action on hematopoietic stem cells is included in the article by Marsh (9). More recent studies have shown that a high dose (150 mg/kg) eliminates all proliferating committed cells and spares just a noncycling stem cell with a very high repopulating ability (10). If this stem cell is not virus-infected and enables a rapid regeneration, the effect on the Friend leukemia and the Ep-independent erythropoiesis should be similar to that described for hydroxyurea treatment.

MATERIALS AND METHODS

Virus

N-tropic polycythemia-inducing Friend murine leu-

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[1] Received Dec 30, 1981; revised July 9, 1982; accepted July 26, 1982.

[2] Supported by the Deutsche Forschungsgemeinschaft (SFB 112).

[3] Department of Clinical Physiology, University of Ulm, W Germany.

[4] We thank E. Barthel and K. Steinhoff for their technical assistance.

**Reprint requests to:* Dr. H. J. Seidel, Department of Clinical Physiology, University of Ulm, Oberer Eselsberg, 7900 Ulm, W Germany.

kemia virus (F-MuLV-P) was provided by R. A. Steeves (Albert Einstein Medical College, New York). It was serially passaged ip in DBA/2 mice with 0.25 ml of a 10% cell-free homogenate of leukemic spleens (2–3 g) in saline.

Mice

Female DBA/2 and BDF$_1$ mice (Gl. Bomholtgard, Ry, Denmark) weighing 19–22 g were used. The mice were kept ten to a cage in artificial light for 12 hrs daily. They were fed commercial pellets and water ad libitum.

Experimental Procedures

For each experiment, four to six mice were used. The mice were killed by cervical dislocation, their spleen weights were determined, and a single-cell suspension of pooled spleen or femur marrow was prepared in α-medium (Flow Laboratories, Inc, Rockville, Md) containing 2% fetal calf serum (Seromed, Munich). Appropriate cell concentrations were obtained by dilution and the cells were counted in a Coulter counter. The total spleen cellularity was calculated according to Wendling et al (11), 100 mg corresponding to 10 × 10^7 cells.

Blood for hematologic studies was taken from the retro-orbital sinus and studied by standard procedures. Reticulocytes were stained with new methylene blue and counted.

CFU-S Experiments

The procedure described by Till and McCulloch (12) was used to assay multipoint hematopoietic stem cells. The pooled marrow or spleen cells were injected iv (2–6 × 10^4 marrow cells and 2–6 × 10^5 spleen cells in 0.25 ml) into irradiated mice (ten animals/group). Irradiation consisted of an 800-rad exposure at 280 kV and 12 mA, with a 1.4-mm Cu and 1-mm Al filter, a focal distance of 50 cm, and a rate of 30 R/min. The recipients were killed 9 days after injection of cells, and their spleens were removed and placed in Bouin's solution in preparation for counting macroscopic colonies after 24 hrs in the fixative. From these data, the mean CFU-S content per femur or spleen was calculated. No endogenously formed colonies were found in mice exposed to radiation but not grafted with cells.

CFU-C Experiments

Cells from bone marrow or spleen were incubated in 3.5-cm plastic dishes containing 1 ml of α-medium with 20% horse serum (Seromed) and 0.3% agar. Colony growth was stimulated by the use of heat-inactivated serum obtained from NMRI mice 3 hrs after they received iv injections of 50 μg of endotoxin (*Salmonella abortus equi;* Difco Laboratories, Detroit). Optimal stimulation was obtained by the addition of 12.5 or 25 μl of endotoxin-activated serum. The cell number per plate was 0.4–2 × 10^5 for bone marrow cells and 0.5–2 × 10^6 for spleen cells. The cultures were done in triplicate and incubated in 5% CO_2 at 37°C in a humidified atmosphere. After 7 days of incubation, the colonies (> 50 cells being a colony) were counted with the use of 40-fold magnification. In each experiment, a control culture with normal bone marrow and spleen cells was included. The number of colonies per dish varied about 10% of the mean.

CFU-E Experiments

The method described by Iscove and Sieber (13) was used. The following were mixed in a final volume of 2.5 ml: 0.8% methylcellulose; 30% fetal calf serum; Ep step III (Connaught Medical Research Laboratories, Toronto), 0.2 or 0.3 units/ml, depending on the batch; α-thioglycerol at an end concentration of 10^{-4} M; and bone marrow or spleen cells in α-medium. Then 1 ml with 3 × 10^5 cells was plated into a 3.5-cm plastic petri dish (Greiner & Co, Nürtingen, Federal Republic of Germany). For each experiment, four parallel cultures were set up. Colonies were counted after 48 hours' incubation at 37°C in a humidified atmosphere containing 5% CO_2. Erythroid colonies with more than eight small cells (erythroblasts) were scored without staining at an 80-fold magnification.

Cultures from F-MuLV-P-infected mice were also set up without addition of Ep, in order to see the F-MuLV-P-specific Ep-independent CFU-E growth (1–3). Ep independence of the complete erythroid precursor compartment was identified when the same number of colonies was seen in the plates with and without addition of Ep and filled with the same cell suspension from an F-MuLV-P-infected animal.

The variation in the number of colonies per plate filled with the same cell suspension did not exceed 10%. As in the controls, day-to-day variations in the yield of erythroid colonies were found, generally dependent on the different batches of methylcellulose used and changes in pH during the preparation of the cell suspension and cultivation in the incubator. Therefore, parallel cultures from uninfected controls were plated for each experiment.

5-FU Treatment

5-FU (Hoffmann-La Roche) was dissolved in saline and the doses indicated were injected ip after dilution in 0.2 ml.

RESULTS

Effect of 5-FU Treatment on Spleen Weight and Reticulocytosis

Groups of DBA/2 mice were infected with F-MuLV-P and received a single 37.5–200-mg/kg dose of 5-FU 12 days later. Spleen weight and reticulocytosis were reduced as shown in figures 1 and 2, respectively. There was a clear dose-dependent effect, with a progressive recovery of both parameters from Day 7 on. After a dose of 37.5 mg/kg, the regeneration was almost completed at this time. In addition, bone marrow cellularity and hematocrit were determined. Bone marrow cellularity recovered parallel to the increase of the spleen weight. The hematocrit of untreated F-MuLV-P-infected mice rose from 61% at the beginning of the experiment to 77% at Day 14; after 5-FU treatment it fell in a dose-dependent manner to 44%–57% at Day 7 and 40%–76% at Day 14.

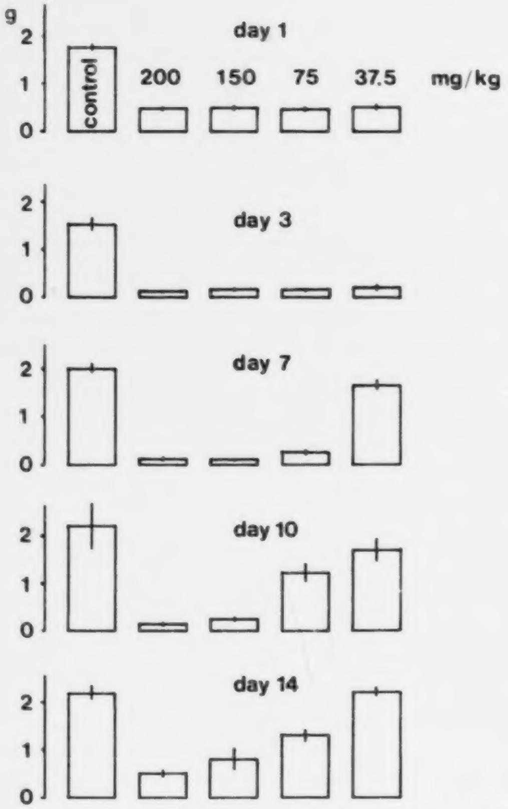

FIGURE 1.—Effect of a single dose of 5-FU ip on spleen weight of F-MuLV-P-infected DBA/2 mice. Treatment was given 12 days after virus infection. Vertical bars indicate SEM.

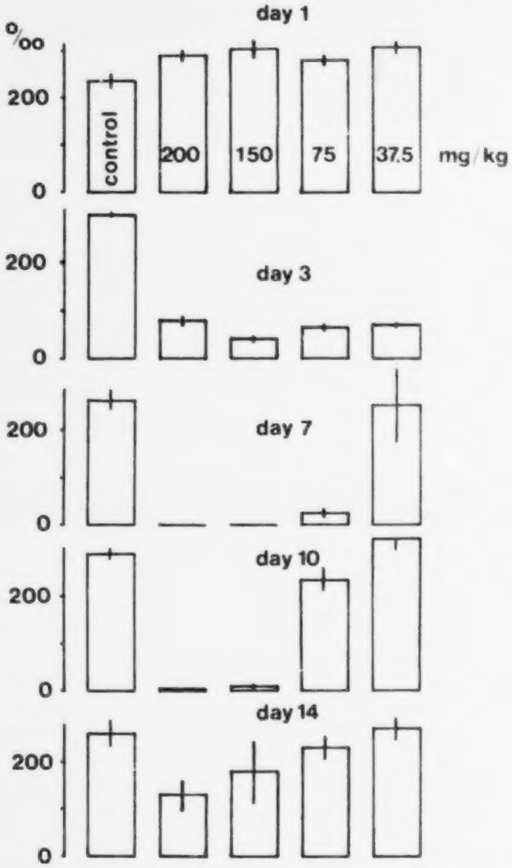

FIGURE 2.—Effect of a single dose of 5-FU ip on reticulocytosis after F-MuLV-P infection.

Effect of Single Doses of 5-FU on Hematopoietic Stem Cells

The effect of a 100-mg/kg ip dose of 5-FU was studied in uninfected and F-MuLV-P-infected mice (table 1). As seen from the same numbers of CFU-E with or without addition of Ep, the erythroid system in the bone marrow and spleen was completely Ep-independent in the F-MuLV-P-infected groups that were not given 5-FU (this was also the case in all other experiments and will not be mentioned further). After treatment, CFU-E were completely eliminated from the femur and the spleen by Day 1 in virus-infected mice and by Day 3 in both groups. The CFU-E seemed to be more sensitive in virus-infected mice. By Day 5 the erythroid regeneration had started in the femur and the spleen of uninfected and virus-infected mice. In the femur of these mice, no colonies were seen without addition of Ep; in

TABLE 1.—Effect of 100 mg/kg of 5-FU ip on CFU-C and CFU-E in femur and spleen of uninfected mice and those infected 12 days previously with F-MuLV-P

Days after 5-FU treatment	Study group	Bone marrow				Spleen			
		No. of cells/femur (× 10^6)	No. of CFU-C/femur	No. of CFU-E/femur		Weight (mg)	No. of CFU-C/spleen	No. of CFU-E/spleen	
				− Ep	+ Ep			− Ep	+ Ep
1	Uninfected	15.4	10,270	—	17,400 ± 520	109 ± 7	1230	—	29,340 ± 1090
	+ 5-FU	10.7	200	—	750 ± 200	100 ± 5	100	—	0
	F-MuLV-P	12.5	8910	5625 ± 370	5125 ± 310	2240 ± 240	21,700	4.8 × 10^6	5.4 × 10^6
	+ 5-FU	9.5	0	0	0	830 ± 135	0	0	0
3	Uninfected	14.3	17,920	—	13,440 ± 280	94 ± 19	3050	—	29,300 ± 1000
	+ 5-FU	3.9	810	—	0	98 ± 10	80	—	0
	F-MuLV-P	10.2	13,700	820 ± 290	1120 ± 200	1285 ± 180	122,980	3.3 × 10^6	3.1 × 10^6
	+ 5-FU	3.5	70	0	0	181 ± 27	0	0	0
5	Uninfected	16.8	18,820	—	13,270 ± 1000	121 ± 9	—	—	20,670 ± 4600
	+ 5-FU	3.9	30	—	1290 ± 120	101 ± 22	710	—	12,110 ± 1640
	F-MuLV-P	11.3	–	3050 ± 380	2710 ± 320	1700 ± 420	69,070	4.7 × 10^6	4.7 × 10^6
	+ 5-FU	1.8	840	0	120 ± 90	143 ± 28	3450	17,120 ± 2310	15,690 ± 2730

the spleen there was no significant difference in the colony number with or without Ep.

In the next experiment, 37.5 and 75 mg/kg were used and the CFU-C and CFU-E of the femur were measured in uninfected and F-MuLV-P-infected mice (table 2). There was a dose-dependent reduction and regeneration of CFU-C and CFU-E. Normal CFU-E (those requiring the addition of Ep) were seen in F-MuLV-P-infected mice at Day 3 after the lower 5-FU dose and in very small numbers at Day 5 after the higher dose. Furthermore, the reduction of the CFU-C and CFU-E numbers was more pronounced in the virus-infected mice.

Multiple Doses of 5-FU

A series of experiments was then done with 18.75-, 37.5-, and 50-mg/kg doses of 5-FU and also with multiple doses of 5-FU given at 12- or 24-hr intervals. In all experiments, the parameters were studied as described before, but the results are presented in a shorter form (figs 3 and 4). In all experiments, the daily control for F-MuLV-P-infected mice (CFU-E per femur) varied to the same extent as in tables 1 and 2 and was set as 100% for each day. CFU-E numbers in 5-FU-treated mice were related to this daily control, and the colonies grown with and without Ep were given in percent. The two lower doses of 5-FU were followed by an overshoot in CFU-E per femur (fig 3). By Days 4–6, all groups had both Ep-dependent and Ep-independent CFU-E; the latter showed a relative increase with time. After two injections of 18.7 mg/kg at 24- or 12-hr intervals, the majority of CFU-E seen at Days 3 and 4 after treat-

TABLE 2.—Effect of 37.5 or 75 mg/kg of 5-FU ip on CFU-C and CFU-E in femur and spleen of uninfected mice and those infected 12 days previously with F-MuLV-P

Days after 5-FU treatment	5-FU dose (mg/kg)	Uninfected mice			F-MuLV-P-infected mice			
		No. of cells/femur (× 10^6)	No. of CFU-C	No. of CFU-E	No. of cells/femur (× 10^6)	No. of CFU-C	No. of CFU-E	
							− Ep	+ Ep
3	Untreated	15.2	30,600*	10,490 ± 380	11.3	18,570	11,640 ± 380	10,850 ± 660
	75	5.4	2160	5350 ± 240	2.6	360	0	0
	37.5	7.9	3160	13,190 ± 450	5.3	0	640 ± 90	1960 ± 200
5	Untreated	16.9	24,790	21,460 ± 880	11.2	14,670	5890 ± 420	5710 ± 210
	75	5.8	20,300†	7420 ± 280	3.1	4960	300 ± 30	510 ± 60
	37.5	12.9	35,130†	13,670 ± 490	7.9	0	12,640 ± 670	13,035 ± 390
7	Untreated	16.0	23,840	22,240 ± 470	11.5	15,840	20,590 ± 390	20,120 ± 600
	75	10.3	12,360	10,400 ± 510	6.8	10,620	5640 ± 540	6120 ± 460
	37.5	11.5	22,340	8510 ± 580	9.2	7270	12,790 ± 370	12,510 ± 330
10	Untreated	13.0	14,470	17,290 ± 605	12.3	12,710	3570 ± 240	3320 ± 230
	75	16.4	18,040	13,450 ± 670	8.8	6930	8540 ± 600	8180 ± 430
	37.5	16.8	16,850	14,280 ± 500	9.8	7690	4900 ± 250	5000 ± 400

*SEM of CFU-C determinations ± 8%.

†Small colonies.

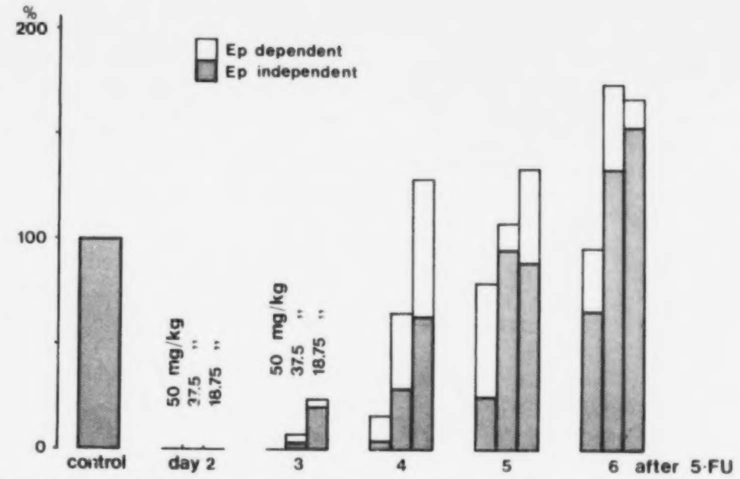

FIGURE 3.—Effect of 5-FU treatment on CFU-E per femur in mice infected with F-MuLV-P 12 days previously. Untreated control groups were studied at each experimental day and the number of CFU-E per femur was set as 100%; all colony growth in these F-MuLV-P-infected mice was Ep-independent.

ment were Ep-dependent (fig 4). But later, at Days 5–7 after two or even three injections of 18.7 mg/kg of 5-FU at 12-hr intervals, all CFU-E growth was Ep-independent again (data not shown).

Effect of 5-FU on Normal and F-MuLV-P-Transformed Erythropoiesis

In some of the experiments described before, the depression of the CFU-E growth was more pronounced in F-MuLV-P-infected mice than in uninfected mice after treatment with 5-FU, marginally so at Day 5 after 100 mg/kg (table 1) and clearly so at Days 3 and 5 after 37.5 and 75 mg/kg (table 2). In a further experiment, single doses of 18.75 and 37.5 mg/kg were studied in uninfected and F-MuLV-P-infected mice (fig 5). The results confirm both observations made in the previous experiments: the induction of a substantial number of Ep-dependent CFU-E and the more pronounced effect of 5-FU in F-MuLV-P-infected mice. After a dose of 37.5 mg/kg, the CFU-E number in uninfected mice did not fall below 10% at Days 1 and 2 and showed an overshoot at Days 3 and 4. CFU-E numbers in infected mice given 37.5 mg/kg were lower and no overshoot was seen, even at Day 4. After a dose of 18.75 mg/kg, the reduction in uninfected mice persisted until Day 4, with a minimum at Day 2 and no overshoot. In infected mice, lower numbers were seen at Days 1 and 2, and they were followed by an overshoot at Day 4.

In the final experiments, uninfected and F-MuLV-P-infected DBA/2 mice were treated with 18.75 or 37.5 mg/kg of 5-FU, and the CFU-S, CFU-C, and CFU-E

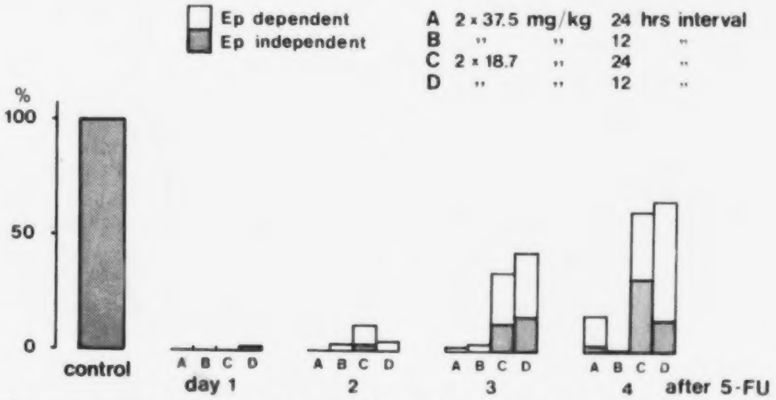

FIGURE 4.—Effect of 5-FU treatment on CFU-E growth per femur, with % calculated as per fig 3.

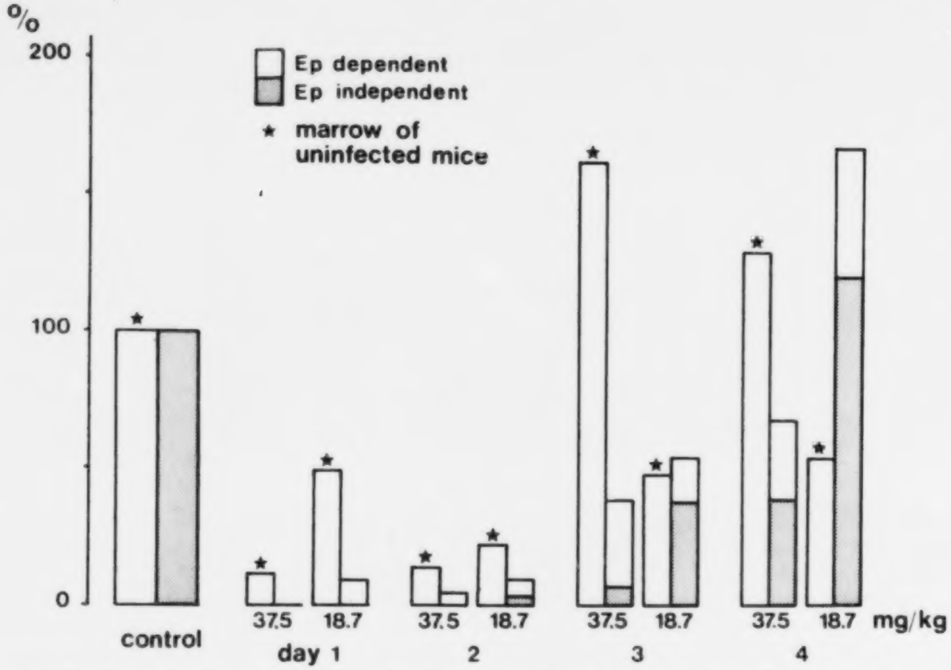

FIGURE 5.—Depression and regeneration of CFU-E numbers in the femur of uninfected (with stars) and F-MuLV-P-infected DBA/2 mice after treatment with 18.75 or 37.5 mg/kg of 5-FU. The % was calculated as per fig 3.

were studied at Days 1, 2, 4, and 7. The effect on the CFU-E compartment was as in the previous experiments, with an overshoot at Day 4 and > 50% Ep-dependent CFU-E after both doses; by Day 7 all CFU-E growth was Ep-independent again. The CFU-S and CFU-C data of Days 1 and 2 after 37.5 mg/kg are included in table 3, which shows lower values at Day 2 for all stem cell types from infected mice compared to values for uninfected controls.

DISCUSSION

Our results show that this viral leukemia can be temporarily suppressed by 5-FU and brought into a remission in the sense that Ep-dependent normal erythropoiesis is induced. This was also seen during regeneration after hydroxyurea treatment (6). Both drugs preferentially kill cells in S-phase, but the cytotoxicity of 5-FU in higher doses is not limited to this part of the cell cycle, as determined from many reports of the high kill of CFU-S in normal uninfected mice [(9,14–16), present study]. Such a treatment should be advantageous, since F-MuLV-P infection is thought to be restricted to cells in active turnover and there is strong evidence that the hematopoietic progenitor cells must be in cycle in order to be transformed by the virus (17–20).

TABLE 3.—Effect of a single dose of 37.5 mg/kg of 5-FU on hematopoietic stem cell regeneration in uninfected controls and mice infected 12 days previously with F-MuLV-P

	Study group	Regeneration after 5-FU*		No. of cells/femur
		Day 1	Day 2	
CFU-S	Uninfected	34	32	5400 ± 300
	F-MuLV-P	34	17	3500 ± 200
CFU-C	Uninfected	5	60	16,500
	F-MuLV-P	6	4	12,000
CFU-E	Uninfected	22	42	22,000 ± 400
	F-MuLV-P	8	0	500 ± 300

*Values are % of values in untreated mice.

The results indicated that high doses of 5-FU are followed by a rather late regeneration of CFU-E, even later in infected mice than in uninfected mice. The CFU-E that were present at Days 5 and 7 after doses of 37.5, 75, and 100 mg/kg were all Ep-independent. Lower doses were followed by an earlier regeneration, and Ep dependency was seen, again followed by Ep independency at Day 5–7. These data suggest the following interpretation: 5-FU kills proliferating cells in a dose-dependent manner and regeneration starts from surviving pluripotent stem cells that were in G_0 and not infected. In the case of a high kill, regeneration is slow and consequently cells reaching the target cell level were infected completely. After a lower cell kill, regeneration is faster, comparable to the recovery after multiple doses of hydroxyurea, and consequently a first erythroid wave occurred before infection took place.

The second phenomenon observed concerns the longer depression of stem cells in virus-infected animals compared to uninfected mice. This agrees with a higher turnover of these cells in F-MuLV-P-infected mice. Data in the literature on CFU-S show a higher percentage in DNA synthesis, but a variation seems to exist (21,22). Our own unpublished data using hydroxyurea (2 hrs after 900 mg/kg) or the ^{3}H-thymidine suicide method showed kill rates of 5%–63% for CFU-S, compared to 10%–15% for controls. The corresponding values for CFU-E were 70% and 90% for infected and uninfected mice, respectively. 5-FU treatment has been suggested as a suitable tool for the study of cell turnover, exceeding the determination of the fraction of a cell population in S-phase (23), and the data presented seem to illustrate this possibility.

This paper finishes our series on the effect of cytotoxic drugs on hematopoietic stem cells in Friend leukemia. The series has included drugs with different modes of action, although they all led to a reduction in the spleen size: busulfan (4), with its major activity on low-cycling CFU-S; dactinomycin (4,5), with an almost selective effect on the erythroid cell system including CFU-E; carmustine (24), an alkylating agent; hydroxyurea (6); and now 5-FU. As discussed, only after hydroxyurea and 5-FU treatment could a short period be seen where most of the detectable CFU-E were normal again. This indicates that the F-MuLV-P-infected stem cells are rapidly cycling cells that can be eliminated, and that the regeneration of normal erythropoiesis starts from an uninfected precursor compartment. After some days, however, the virus causes a rapid transformation. The regeneration of Ep-independent erythropoiesis after dactinomycin treatment has been explained by the persistence of virus-infected precursors, most probably early erythroid-committed stem cells, BFU-E (5). Busulfan and carmustine, in this experimental system, are too toxic for the cell compartments that are responsible for the hematopoietic regeneration. Therefore, the erythroid system regenerated more slowly and was immediately Ep-independent after treatment, either due to the survival of some infected precursors or by reinfection.

In conclusion, this viral leukemia can be suppressed and temporarily reversed by drugs with a preferential action on proliferating cells; it cannot be cured by these, however, due to the persistence of the virus. Antiviral chemotherapy either alone or in combination with hydroxyurea was without success (25,26). In view of these results, we hope that the findings presented in this study contribute to the understanding of the hematopoietic compartments in an experimental leukemia. With respect to therapeutic success, it is obvious that chemotherapy of this virus-induced malignancy, even with the most effective drugs, has to be combined with other approaches, especially immunologic ones such as those presented for Friend leukemia by Schäfer et al (27) and in more recent studies (28).

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Studies of the Pharmacology of *N*-Methylformamide in Mice[1,2]

Charles Brindley, Andreas Gescher,* Ernest S. Harpur, David Ross, John A. Slack, Michael D. Threadgill, and Helen Whitby[3,4]

When 400 mg/kg of ^{14}C-methyl-labeled *N*-methylformamide (NMF) was injected ip into mice, the curve for plasma concentration of radioactivity versus time was superimposable on the curve obtained by measuring unmetabolized NMF with gas-liquid chromatography during the first 24 hrs. Radioactivity in plasma was measurable for 8 days after NMF administration, but NMF was not measurable by gas chromatography beyond 24 hrs after administration. Radioactivity was eliminated from the plasma after 60 hrs, with an apparent half-life of 71.1 hrs. Of the radioactivity injected with NMF, 73.6% was recovered in the urine in 24 hrs; 26.4% of this was unchanged NMF. Three percent of the administered radioactivity was exhaled as $^{14}CO_2$ in 7 hrs at a constant rate of 0.007% per min. One urinary metabolite was a stable precursor of formaldehyde, which decomposed to formaldehyde only after alkaline hydrolysis and may well be *N*-(hydroxymethyl)-formamide. The areas under the plasma concentration versus time curve were estimated after ip, iv, and oral administration of NMF. The bioavailability of NMF was 1.01 after oral administration and 1.10 after ip administration. [Cancer Treat Rep 66:1957–1965, 1982]

The antitumor activity of *N*-methylformamide (NMF) (OHC-NHCH$_3$) against murine tumor models was first described by Clarke et al in 1953 (1). In a clinical trial, NMF was found to cause reversible liver damage in all seven patients in whom it was tested (2). We have recently found NMF to be active against the mouse ovarian reticulum cell sarcoma M5076 and the mouse TLX5 lymphoma (3). The animal tumors that are sensitive to NMF do not respond to treatment with other *N*-alkyl derivatives of formamide or with formamide itself.

The mechanism by which NMF exerts antitumor activity is unknown. We found NMF to be cytotoxic only at concentrations > 0.5 M against TLX5 lymphoma cells in an in vitro-in vivo bioassay (3). In this bioassay, *N*-ethylformamide (the ethyl analog of NMF), which is devoid of antitumor activity in vivo, exhibited cytotoxicity comparable to that of NMF.

Since NMF possesses antitumor activity against a considerable number of murine tumors and does not appear to be toxic to the bone marrow (4), it is a candidate for further clinical evaluation. To gain information that may facilitate the rational use of NMF in clinical trials, several aspects of its disposition in the mouse were studied. ^{14}C-Methyl-labeled NMF (^{14}C-NMF) was synthesized and the plasma disposition of radioactivity was compared with that of unlabeled NMF.

Renal excretion and metabolism to CO$_2$ are two obvious routes of elimination that a small molecular weight compound like NMF can undergo. Therefore, the appearance of radioactivity after administration of ^{14}C-NMF was studied in the urine and the breath. Special attention was given to identifying products of the oxidative metabolism of NMF in the urine, in view of our finding that formamide is a urinary metabolite (3).

The bioavailability of NMF after ip or oral administration was also investigated because in the antitumor tests, NMF was administered ip, whereas in the clinic, NMF may be given orally as well as iv or im. Finally, the influence of the presence of the TLX5 lymphoma on the plasma disposition of NMF was investigated.

MATERIALS AND METHODS

NMF and formamide were purchased from Aldrich Chemicals (UK); *N*-(hydroxymethyl)-formamide was synthesized by Mr. E. N. Gate in our laboratories ac-

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[1] Received Mar 30, 1982; revised July 23, 1982; accepted Aug 16, 1982.

[2] Supported by grants from the Medical Research Council and the Cancer Research Campaign of Great Britain.

[3] CRC Experimental Chemotherapy Research Group, Department of Pharmacy, University of Aston, Birmingham, England.

[4] We thank Dr. J. A. Hickman for his interest in this work and Karen Ensor for her help with the chromatographic determination.

* *Reprint requests to:* Dr. A. Gescher, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, England.

cording to a published method (5). ^{14}C-NMF was prepared by treatment of ^{14}C-methylamine hydrochloride (Radiochemical Centre, Amersham, UK) with ethyl formate and sodium carbonate; details of the synthetic procedure will be published elsewhere. Drugs were administered in solution in sterile saline. Male CBA CA mice (20–25 g) were used throughout this study. Approximately 10^5 TLX5 cells from routine passage as ascites were inoculated sc in the inguinal region of 20-g mice.

Measurement of NMF and total radioactivity in the plasma and urine.—The drug was injected into a tail vein iv, administered ip, or given by esophageal intubation. The doses administered were 400 mg/kg (equivalent to 1/2 × LD10 determined by ip administration) and 80 mg/kg. The amount of radioactivity injected into each mouse with the dose was either 5 or 15 μCi. While mice were under halothane anesthesia, blood samples (20 μl) were collected into heparinized capillary tubes from the tip of the tail for at least ten time intervals following drug administration. In a control experiment, blood was obtained without anesthetizing the animals, and plasma concentration versus time profiles were not different from profiles derived from anesthetized animals. Plasma was obtained by centrifugation, and samples were diluted with acetone and centrifuged again. Urine was collected from mice kept in metabolic cages (Jencons, UK). To minimize the risk of evaporative losses, the urinary collection funnels were washed with 10 ml of water after 2–6-hr intervals during the first 12 hrs and again 24, 48, and 72 hrs after drug administration. For the analysis by gas chromatography (GC), 10 μl of blood or 100 μl of diluted urine was further diluted with 50 or 200 μl of acetone (including internal standard). Radioactivity was counted after adding 10 μl of blood or 1 ml of diluted urine to 5 or 10 ml of NE260 Micellar scintillant (New England Nuclear, W Germany), in a Packard Tricarb 2660 scintillation counter using the external standardization mode. The centrifuged plasma or urine samples containing acetone with tetramethylurea as internal standard were analyzed by GC as described previously (3). The limit of detection for NMF was 3–5 μg/ml.

Breath analysis.—After iv administration of ^{14}C-NMF, the mice were placed in air-tight metabolic cages and the exhaled radioactivity was collected continually in a trapping fluid that was renewed at frequent intervals as previously described in reference 6. The trapping fluid did not contain measurable amounts of unmetabolized NMF as indicated by GC analysis, so that most of the exhaled radioactivity was considered to be in the form of $^{14}CO_2$.

Pharmacokinetic analysis.—The decline in plasma concentration of radioactivity with time beyond 24 hrs after drug administration was multiexponential. The decline of plasma concentration of radioactivity beyond 60 hrs after administration of ^{14}C-NMF was considered to represent the elimination of drug or metabolites, and in each animal the plasma concentration values obtained beyond 60 hrs after ^{14}C-NMF administration were subjected to a linear regression analysis that afforded a line (correlation coefficient > 0.98) from which a half-life was computed. The areas under the plasma concentration versus time curves (AUC) for unchanged NMF up to 24 hrs after NMF administration were estimated by the trapezoidal rule. AUC and half-life values as well as recoveries of NMF and radioactivity in the urine, as given in the Results section, are the mean ± SD from at least five animals. The bioavailability was calculated, using mean AUC data, from the ratio $\frac{\text{AUC ip or oral}}{\text{AUC iv}}$.

Analysis of formaldehyde precursors in the urine.—Urine samples were diluted and made alkaline by the addition of 1 N NaOH (to pH 12), and liberated formaldehyde was measured colorimetrically according to Nash (7).

RESULTS

NMF (400 mg/kg) was injected ip and its concentration in plasma was measured by GC and compared with the plasma disposition of radioactivity after administration of ^{14}C-NMF. Figure 1 shows that the plasma concentration versus time plot of NMF as measured by GC is very similar to that of total radioactivity during the first 24 hrs after drug administration. This suggests that unchanged NMF is the main constituent of the total radioactivity measured in the plasma. GC estimation of NMF in plasma samples collected beyond 24 hrs after NMF administration was unreliable since levels were generally near or below the detection limit of the assay. Radioactive species derived from ^{14}C-NMF were, however, measurable in the plasma for 8 days after drug administration (fig 2). The apparent concave downward curvature of the line up to 24 hrs on a semilog plot (fig 1) suggests that the disappearance of the drug from plasma does not obey first-order kinetics during this time. However, the disposition of radioactivity beyond 24 hrs can be described by a curve that appears to exhibit first-order kinetics with at least two exponential components (fig 2). Radioactivity was detectable in the plasma up to 192 hrs after administration of ^{14}C-NMF. The decline of plasma concentration of radioactivity with time between 60 and 192 hrs after drug administration is linear and may well represent the elimination of radioactivity, ie, of NMF and/or its metabolites. (Elimination rate constant K_{el} = 0.0097 ± 0.0012 hr^{-1}; elimination half-life = 71.1 ± 8.8 hrs.)

A total of 73.6% ± 6.8% of the radioactivity was recovered in the urine within 24 hrs after ip administration of 400 mg/kg of ^{14}C-NMF. Figure 3 shows that

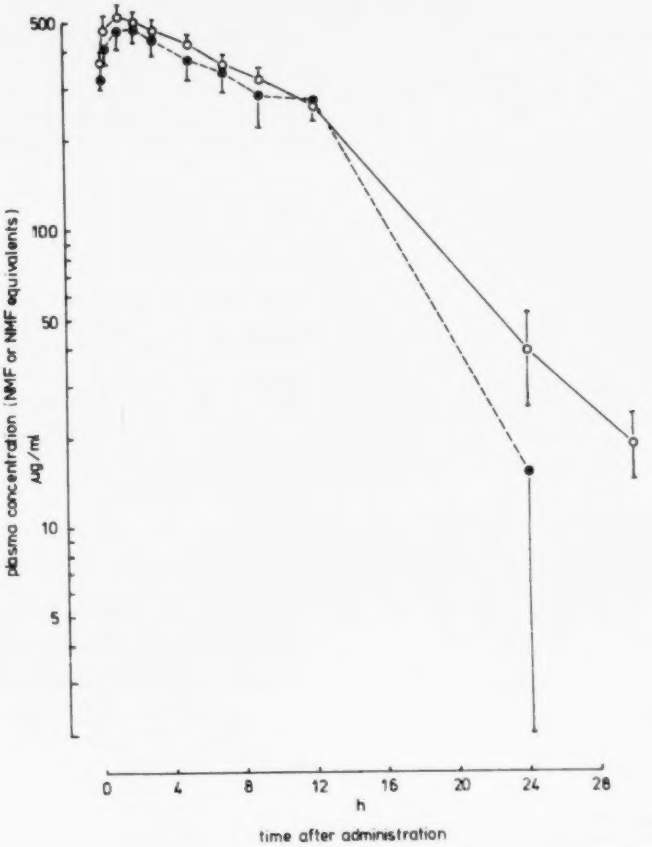


FIGURE 1.—Plasma disposition of NMF measured by GC (●) and of total radioactivity (○) after ip administration of 400 mg/kg of NMF or ^{14}C-NMF. Concentrations of radioactivity are expressed as NMF equivalents. Values are the mean ± SD of 6 experiments, except for the point without an error bar, which is the mean of 3 experiments.

26.4% ± 2.9% of the radioactivity in the urine was unchanged NMF and < 2% (not shown in fig 3) of the dose was eliminated as either formamide, the *N*-demethylated metabolite of NMF, or its *N*-hydroxymethyl precursor, *N*-(hydroxymethyl)-formamide ($OHCNHCH_2OH$); the latter also appears as formamide on GC determination. Some urinary metabolites (or a metabolite) of NMF were hydrolyzed to formaldehyde when urine samples were treated with NaOH, and the hydrolysate reacted with Nash reagent. In two experiments, 4.4 µmols of the metabolites (expressed as formaldehyde equivalents) were excreted with the urine in 36 hrs. *N*-(Hydroxymethyl)-formamide releases formaldehyde on alkaline hydrolysis, so the urinary formaldehyde precursor may well be this carbinolamine. Control animals and those that received *N*-ethylformamide did not excrete formaldehyde precursors sensitive to alkali.

Radioactivity exhaled with the breath as $^{14}CO_2$ after administration of 400 mg/kg of ^{14}C-NMF was collected for 7 hrs. Only 3% of the radioactivity injected was exhaled during this time, at a constant rate of 0.007%/min.

When NMF was administered iv or orally, the disappearance of drug from plasma with time did not substantially differ from the disappearance of NMF from the plasma after ip injection. In figures 4 and 5, graphs of NMF plasma concentrations plotted on a linear scale against time after administration of two iv (fig 4) and oral (fig 5) doses of NMF (80 and 400 mg/kg) reveal apparently linear sections, which indicate that zero-order kinetics might partially govern these plasma disposition profiles. The time required for plasma levels to decline from peak concentrations to 50% after oral administration of NMF (fig 5) was 9.5 hrs for the high dose and 8 hrs for the low dose.

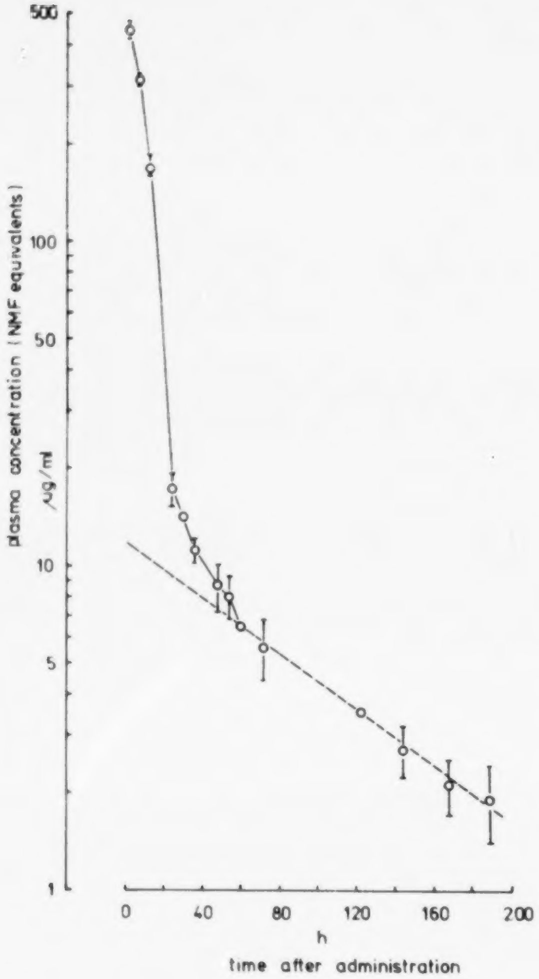


FIGURE 2.—Plasma disposition of radioactivity after ip administration of 400 mg/kg of ^{14}C-NMF. Values are the mean ± SD of 6 experiments; the values without error bars are the mean of 3 experiments. Dashed line indicates the line obtained by linear regression analysis of points beyond 60 hrs after drug administration.

AUC values were calculated up to 24 hrs after administration of 400 mg/kg of NMF and were 5235 ± 1270 $\mu g \cdot hr \cdot ml^{-1}$ after iv administration, 5842 ± 783 $\mu g \cdot hr \cdot ml^{-1}$ after ip administration, and 5360 ± 1117 $\mu g \cdot hr \cdot ml^{-1}$ after oral administration. Thus, the systemic availability of NMF was 1.01 after oral administration and 1.10 after ip injection.

Mice were inoculated with the TLX5 lymphoma, which is sensitive to NMF (3). Five days after tumor implantation (approximately half the lifespan of untreated tumor-bearing mice), a plasma concentration versus time profile was determined after ip injection of NMF in these mice. It was virtually identical with the profile seen in normal mice. The AUC calculated from the profile in six tumor-bearing mice was 6595 ± 564 $\mu g \cdot hr \cdot ml^{-1}$ and did not differ significantly from the value obtained in normal mice.

DISCUSSION

Radioactivity injected as a single dose of ^{14}C-NMF (400 mg/kg) was detected in plasma for 8 days after drug administration, and the plasma radioactivity concentration versus time curve (fig 2) shows that a drug disposition phase that can be described by first-order kinetics was not reached until 24 hrs after NMF administration. After 60 hrs, radioactivity was cleared from the plasma with an apparent half-life of 71.1 hrs.

The physicochemical nature of NMF and its potential metabolites has so far prevented the unequivocal identification of the radiolabeled species present in this late phase of NMF disposition; the high water:octanol partition coefficient of NMF and its likely metabolites (unpublished results) prevents efficient extraction into organic solvent and their high volatility renders freeze-drying of samples inappropriate.

Repeated daily administration of high doses of NMF was required for optimal antitumor activity in murine tumor models (3), and it is likely that to be effective in patients, NMF may have to be given in high doses on multidose schedules or by constant infusion. If the long elimination half-life of drug-derived species observed in mice (fig 2) is also found in man, then repeated dosing at frequent intervals will lead to drug accumulation. Whether accumulation of NMF or its metabolites is a prerequisite for its antitumor efficacy or the generation of its hepatotoxicity remains to be elucidated.

The plasma disposition profile of radioactivity during the first 24 hrs after drug administration is virtually superimposable on that of unlabeled NMF (fig 1), which shows that only a small amount of metabolite(s), if any, appears in the plasma during this time. However, metabolites were found in the urine (fig 3), which suggests that after their formation, the metabolites (whatever their chemical nature) are rapidly eliminated into the urine.

The plasma concentration versus time curves of NMF (as measured by GC) within 24 hrs after ip (fig 1), iv, and oral administration of 400 mg/kg exhibit a concave downward curvature when plotted semilogarithmically. This curvature was not seen in a preliminary study of NMF plasma levels (3) when blood was obtained by cardiac puncture. The mathematical treatment of these data, based on linear kinetics, might not be appropriate and the concept of plasma elimination rates and half-lives cannot be used to describe these curves. When plotted using Cartesian coordinates, concentration val-

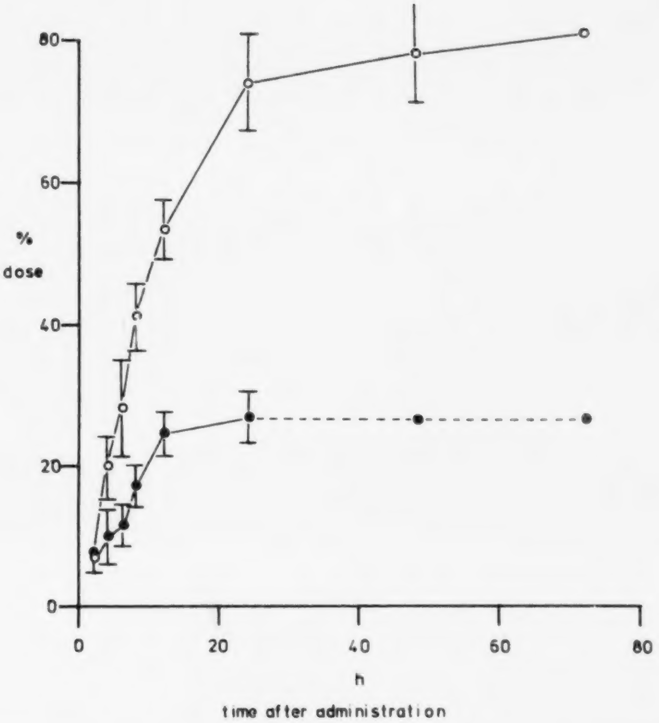


FIGURE 3.—Cumulative urinary excretion of NMF measured by GC (●) and of total radioactivity (○) after ip administration of 400 mg/kg of NMF or ^{14}C-NMF, expressed as % of dose administered. Values are the mean ± SD of 5 experiments. There were no measurable levels of unchanged NMF in the urine after 24 hrs.

ues during some periods of the disposition appear to fit a straight line (figs 4 and 5), which indicates that NMF might be eliminated at a fixed rate independent of its concentration in the plasma (zero-order kinetics). Nonlinear pharmacokinetics are often associated with the saturation of the pathways by which a drug is distributed in or eliminated from the body, eg, uptake into tissues or hepatic metabolism (8). The data in figs 4 and 5 suggest either that both doses are above the saturable point or that the removal process is zero-order regardless of the dose in the first 24 hrs. When plasma concentration values obtained within 24 hrs after administration of 80 and 400 mg/kg of NMF are divided by the dose and the ratios are plotted against time (fig 6), the resulting curves, although not superimposed, are not conspicuously different from each other [nonlinear kinetics must be suspected if the curves are not superimposable (8)]. This does not facilitate a conclusion as to whether or not the plasma disposition of NMF is governed by nonlinear kinetics. No further attempt has yet been made to determine which model best describes the plasma disposition of NMF. Further studies are required to unambiguously establish nonlinearities in the pharmacokinetics of NMF. To define the kinetic model for the disposition is important because it may allow the prediction of drug levels in the body and, as a consequence, the prediction of therapeutic or unwanted drug effects.

Most of the radioactivity injected with NMF was excreted into the urine within 24 hrs (fig 3). Only 26.4% of the dose appeared in the urine as unchanged NMF and < 2% as formamide [or *N*-(hydroxymethyl)-formamide]. To identify the urinary metabolite(s) of NMF is relevant in view of the suggestion that NMF may require metabolic activation to exert its antineoplastic activity (3). Since the metabolite(s) were cleared from

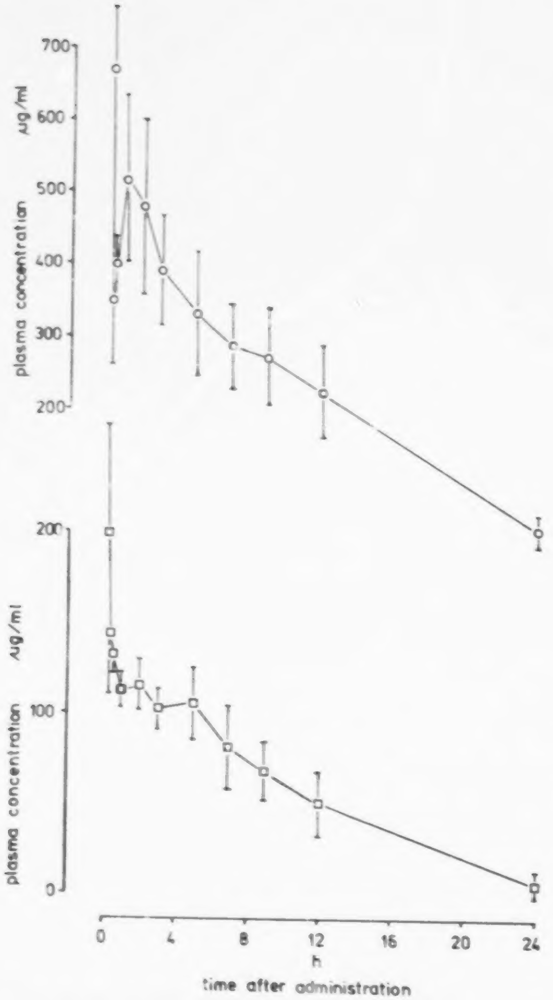
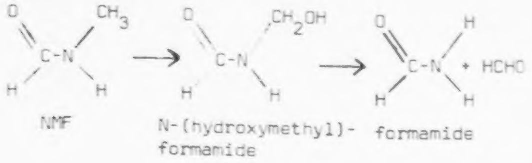


FIGURE 4.—Plasma disposition of NMF after iv administration of 80 mg/kg (□) or 400 mg/kg (○) of NMF. Values are the mean ± SD of at least 5 mice.

the blood rapidly, it is possible that they are very hydrophilic conjugates. Indeed, on alkaline hydrolysis, urine of NMF-treated mice contained a large amount of thiol compounds, suggesting the presence of metabolites with thioether structure, such as *N*-acetylcysteine derivatives (A. Gescher, unpublished observation). One metabolite was characterized as a stable precursor of formaldehyde, presumably *N*-(hydroxymethyl)-formamide. Preliminary unpublished results show that this species does not possess the antitumor efficacy of NMF and does therefore not appear to be an active metabolite. The evidence for the presence in urine of *N*-(hydroxymethyl)-formamide as a metabolite of NMF suggests the following metabolic scheme:



It is puzzling that radioactivity excreted with the breath as $^{14}CO_2$, the final oxidation product of the NMF methyl moiety, was exhaled at a constant rate of 0.007%/min during 7 hrs after drug administration. The $^{14}CO_2$ exhalation pattern observed with ^{14}C-NMF contrasts with the profiles reported after administration of other drugs that undergo metabolic *N*-demethylation, eg, hexamethylmelamine, aminopyrine, and caffeine. In these cases, the $^{14}CO_2$ exhalation profiles reflect the plasma disposition of drug-derived *N*-methyl moieties (6). It is possible that in the case of NMF, the *N*-hydroxymethyl metabolite is so stable that further metabolism (eg, cleavage of the N-C bond to form formaldehyde), since it does not occur spontaneously, is catalyzed by an enzyme that is saturated on administration of large doses of NMF. This might lead to a slow zero-order formation of CO_2, probably circumventing free formaldehyde in the metabolic process, at a rate that is independent of plasma NMF concentration and that in turn determines the rate at which $^{14}CO_2$ appears with the breath.

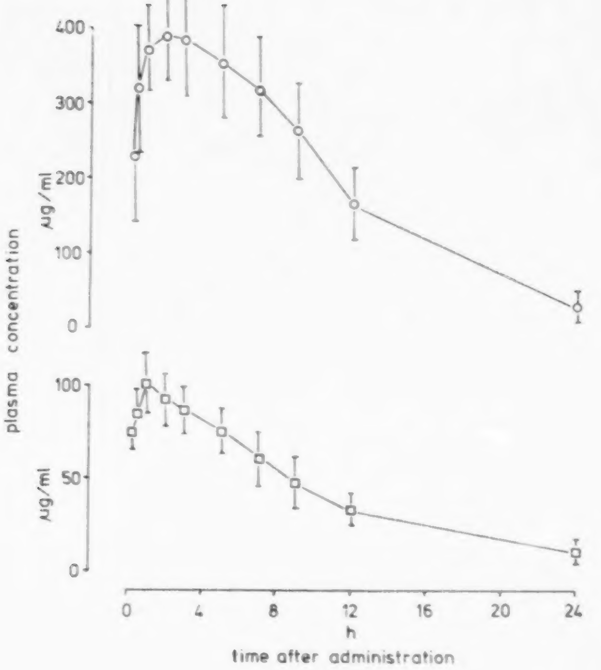


FIGURE 5.—Plasma disposition of NMF after oral administration of 80 mg/kg (□) or 400 mg/kg (○) of NMF. Values are the mean ± SD of 4–8 mice.

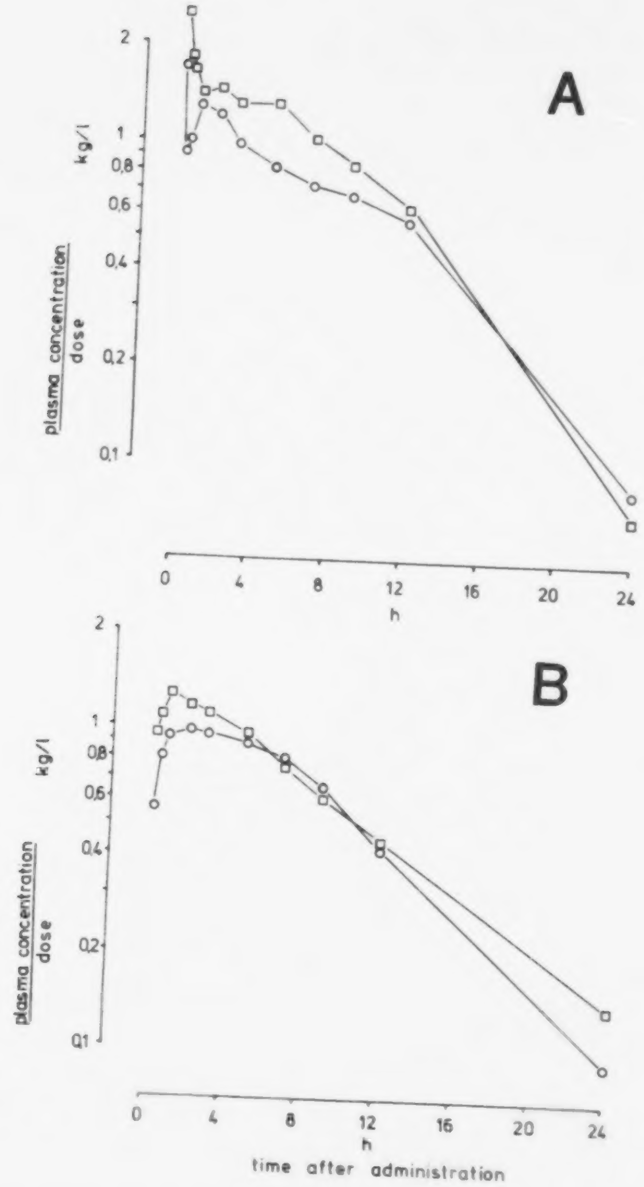


FIGURE 6.—Plots of NMF plasma concentrations divided by the dose against time after iv (A) or oral (B) administration of either 80 mg/kg (□) or 400 mg/kg (○) of NMF. Values are identical with the mean values shown in figs 4 and 5.

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Massive Vincristine Overdose: Failure of Leucovorin To Reduce Toxicity[1]

Lambert L. Thomas,[2,*] **Paul C. Braat,**[2] **Reinier Somers,**[3] **and Robert Goudsmit**[2]

The case history of a 24-year-old male with the nodular sclerosing form of Hodgkin's disease (stage II_E) who accidentally received an overdose of vincristine (25 mg, 0.39 mg/kg) is reported. To date, ten patients given a vincristine overdose have been reported. Two of those patients were treated with leucovorin. One patient died after 68 hours. In the second patient, the time to onset and the duration of symptoms were shortened. In an attempt to reduce the duration of toxic effects in our patient, leucovorin was administered 24 hours after the overdose. In agreement with the earlier observation, the time to onset of symptoms was short, but in contrast we did not find a more rapid recovery from the serious side effects. Although the patient might have died without leucovorin administration, we believe its beneficial effect remains to be established. [Cancer Treat Rep 66:1967–1969, 1982]

CASE REPORT

A few weeks before admission, a 24-year-old male reported a sharp pain in the right hemithorax, radiating to neck and shoulders and aggravated by respiratory movements. His temperature varied periodically between 37.5°C and 38.5°C.

Physical examination disclosed a dull percussion sound at the right sternal border. No other abnormalities were found.

Laboratory investigations showed an erythrocyte sedimentation rate of 40 mm, a leukocyte count of 17.8 $\times 10^9$/L with a normal differential count, and a platelet count of 394 $\times 10^9$/L. Transaminases and other liver enzymes were normal.

An X-ray of the chest showed a large, round shadow in the middle and upper field of the right lung. The shadow had a pyramid-shaped extension in the right upper lobe, with its base on the anterior thoracic wall.

A transthoracic puncture was performed. Cytologic examination of the aspirate showed granulocytes, eosinophils, and numerous Reed-Sternberg giant cells. A supraclavicular lymph node, appearing shortly afterward, enabled the histologic diagnosis of nodular sclerosing type Hodgkin's disease. Since further staging procedures showed no abnormalities, the Hodgkin's disease was classified as stage II_E.

A regimen of three MOPP courses (mechlorethamine, vincristine, procarbazine, and prednisone) followed by mantle field irradiation and completed by three MOPP courses was planned.

By accident, 25 mg of vincristine in 250 ml of supplied diluent was administered in the first treatment regimen. This error was not discovered until the next day. As an attempted prophylactic measure, 12 mg of leucovorin was administered im four times daily for 5 days, starting 24 hours after the vincristine overdose.

The following sequence of events occurred after the overdose (the time course of the development and resolution of symptoms, respectively, is given parenthetically). The patient had nausea, vomiting, and diarrhea (5 hours–4 days). He developed dyspnea, presumably caused by a discrete alveolar-capillary block: pH = 7.50, pCO_2 = 28 mm Hg, pO_2 = 67 mm Hg (10 hours–4 days). There was a short hypotensive period: Riva-Rocci reading = 95/70 and pulse frequency = 140/minute (10 hours–24 hours). Bone marrow depression was present from Day 4 on. The hematocrit, leukocyte count, and platelet count declined to 0.31 (Day 9), 1.7 $\times 10^9$/L (Day 8), and 36 $\times 10^9$/L (Day 6), respectively. On Day 8, segmented leukocytes were virtually absent in the peripheral blood. After a rapid rise, the values were normalized or markedly increased to 0.38, 34.3 $\times 10^9$/L, and 790 $\times 10^9$/L, respectively, on Day 16. In a few weeks, all values were within normal ranges.

The syndrome of inappropriate secretion of antidiuretic hormone (ADH) developed, which promptly responded to fluid restriction (Day 6–Day 10). A urine

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[1] Received Dec 4, 1981; revised Aug 3, 1982; accepted Aug 19, 1982.

[2] Department of Hematology (*L. L. Thomas* and *R. Goudsmit*) and Department of Pulmonology (*P. C. Braat*), University Hospital, Wilhelmina Gasthuis, University of Amsterdam, The Netherlands.

[3] Antonie van Leeuwenhoek Hospital, Amsterdam, The Netherlands.

[*] *Reprint requests to:* Dr. Lambert L. Thomas, Department of Hematology, University Hospital, Wilhelmina Gasthuis, University of Amsterdam, The Netherlands.

sample collected after the nadir of the syndrome showed normal levels of ADH. Parenchymal liver damage was maximal on Day 3, as reflected by elevated SGOT, SGPT, and LDH levels of 128, 208, and 2072 IU/ml, respectively (Day 3–Day 10). Substantial hair loss was noted from Day 12 on. Signs of cerebral dysfunction, eg, anxiety, depression, agitation, and hallucinations, developed after 4 days and gradually declined over several weeks.

The neurotoxicity of the overdose was severe, with paresthesias (Day 2–Day 80) and generalized pain in muscles, jaws, and abdomen (Day 3–Day 8). A complete paralytic ileus developed, necessitating parenteral feeding (Day 5–Day 16). Functional impairment of cranial nerves V, VI, and VIII was noted (Day 4–Day 9). Muscular weakness and loss of tendon reflexes were observed for a longer period (Day 4–Day 80). Bladder atony led to intermittent catheterization (Day 7–Day 16). An electroencephalogram in the acute phase showed no localizing abnormalities but was consistent with diffuse cerebropathy caused by the intoxication.

An impressive tumor regression was observed. The 234-cm^3 mass had virtually dissipated, leaving some ring shadows. A second cytostatic course was given, but without vincristine. No adverse effects were noted. An electromyogram made shortly before discharge showed no remaining signs of polyneuropathy. Six months after the vincristine overdose, neurologic examination still showed a slight paresis of both feet and atrophy of the muscles of both hands. Deep tendon reflexes were absent.

To date, 3 years after diagnosis, there is no evidence of relapse of the patient's Hodgkin's disease.

DISCUSSION

The signs and symptoms of vincristine overdose described in this patient have all been reported previously (1–7). The overdoses have varied from 0.12 to 0.60 mg/kg. Even without an overdose, similar vincristine toxic effects such as peripheral and autonomic neuropathy, cranial nerve palsies, mental changes, and inappropriate ADH secretion are well-known (8). However, the sequelae of a massive overdose are much more serious. Four of the ten reported patients died, the deaths being directly related to vincristine toxicity (1,7). Two patients died of hemorrhagic complications due to bone marrow depression and one died of the complications of a paralytic ileus. The fourth patient died of a disseminated *Candida* infection on Day 24 when he was still profoundly leukopenic.

Maximal supportive care such as prophylaxis against seizures, assisted ventilation when required, blood component therapy during bone marrow depression, and treatment of inappropriate ADH secretion is considered crucial. A patient supported through the critical period may regain normal neurologic function as did our patient (1,6,7), although this may not be the rule (2,4).

Two patients reported were treated with leucovorin, based on the observation of a protective effect of leucovorin in mice given a lethal dose of vincristine (9). One patient died after 68 hours (1); the second recovered completely (6).

Major seizures due to vincristine overdose have been reported (1). Clinical evidence for an exacerbation of epilepsy after administration of folic acid, a structurally very similar molecule, is conflicting in the literature (10), but since our patient had no history of epilepsy we felt justified in administering leucovorin. Grush and Morgan (6) postulated the time to onset and the duration of symptoms to be shorter than those reported in previous publications (1–5,7), as shown in table 1. In agreement with Grush and Morgan, we noted a rather short time to onset of symptoms. However, in contrast, we did not find a more rapid recovery from the serious side effects (table 1).

Although the patient might have died without leucovorin administration, we believe its beneficial effects in vincristine overdose remain to be established.

TABLE 1 —Duration of toxicity in days following vincristine overdose

Toxic effect	Without leucovorin, refs 1–5	With leucovorin	
		Ref 6[*]	Present study[†]
Nausea/vomiting	1–7	1–2	1–4
Neuropathies	3–30	1–29	3–9
Inappropriate ADH	3–16	6–8	6–10
Paresthesias	7–28	7–18	2–80
Bone marrow depression	7–21	5–9	4–16
Gastrointestinal effects	9–21	3–9	3–16
Myopathies	14–60	6–15	4–80
Cerebral dysfunction	14–50	7–8	4–25

[*] Starting 48 hrs after vincristine overdose, 15 mg of leucovorin was given 8 times daily for 3 days.

[†] Starting 24 hrs after vincristine overdose, 12 mg of leucovorin was given 4 times daily for 5 days.

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Combination Chemotherapy for Multiple Myeloma With Melphalan, Prednisone, Cyclophosphamide, Vincristine, and Carmustine (BCNU) (M-2 Protocol)[1]

Umberto Tirelli,[2,*] Diana Crivellari,[2] Antonino Carbone,[3] Andrea Veronesi,[2] Enzo Galligioni,[2] Mauro G. Trovò,[2] Salvatore Tumolo,[2] and Eligio Grigoletto[2,4]

From January 1975 to May 1981, 25 consecutive patients with multiple myeloma (MM) were entered in a prospective study and treated with the M-2 protocol (melphalan, prednisone, cyclophosphamide, vincristine, and carmustine [BCNU]). The Karnofsky performance status was < 70 in 62% of the patients. Nineteen patients were classified as being in stage III, three were in stage II, and three were in stage I. All patients had symptomatic and previously untreated MM. In 17 of 21 (80.9%) evaluable patients, an objective response according to Myeloma Task Force criteria was obtained. There was no difference in response rate among patients in the various stages of the disease or according to types of proteins secreted. The median time to obtain an objective response was 2 months and the median duration of the remission is 18+ months. The actuarial median survival was 42 months. In the first 15 months of follow-up, 20% of the patients died. Toxicity was not negligible and was mainly hematologic. One treatment-related death occurred. Our study confirms the efficacy of the M-2 protocol in MM and supports the data reported by the Memorial Hospital group. [Cancer Treat Rep 66:1971–1973, 1982]

Memorial Hospital investigators claimed that the M-2 protocol (melphalan, prednisone, cyclophosphamide, vincristine, and carmustine [BCNU]) significantly improved the median survival of 46 previously untreated patients with multiple myeloma (MM) in comparison with historical controls (50 versus 22 months) (1). The same authors reported an objective remission rate of 77% according to Myeloma Task Force criteria (2). Surprisingly, few other groups have reported their experience with the M-2 protocol (2). Miller and Perrotta (3) obtained a 71% objective remission rate and a median survival of 21 months in 28 previously untreated patients with MM. Unfortunately, these results were reported in abstract form and no information regarding response criteria and toxicity was provided. Buonanno et al (4) observed three objective responses among nine evaluable patients treated with the M-2 protocol. However, the number of patients, all pretreated, was too small to draw any conclusion. Case (5), one of the coauthors of the two reports of the M-2 protocol at Memorial Hospital (1,6), published in letter form his own results in 20 patients treated between 1976 and 1979 with the M-2 protocol at Maine Medical Center. All patients responded and are alive except two who died from unrelated illness. Most recently, Paccagnella et al (7) reported in abstract form a 78% objective response rate and a median survival of 51 months in 56 previously untreated patients. These results in the largest series reported so far seem to confirm the Memorial Hospital data. However, the relative scarcity of information regarding the activity of the M-2 protocol and the debate about the real impact of the M-2 protocol on survival of patients with MM (5,8) prompted us to report our results with this regimen in a group of patients admitted to a large general hospital.

MATERIALS AND METHODS

From January 1975 to May 1981, 25 consecutive patients with previously untreated and symptomatic MM entered this prospective study. Staging procedures included routine blood analysis, serum and urine immu-

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[1] Received Jan 6, 1982; revised June 21, 1982; accepted June 29, 1982.

[2] Division of Radiotherapy and Medical Oncology, General Hospital, Pordenone, Italy.

[3] Department of Pathology, Istituto Scientifico per lo Studio e la Cura dei Tumori, Genova, Italy.

[4] We thank Drs. Capra and Bassignano, Department of Physics, for statistical analysis.

[*] *Reprint requests to:* Umberto Tirelli, MD, Division of Radiotherapy and Medical Oncology, General Hospital, 33170 Pordenone, Italy.

noelectrophoresis, bone marrow aspiration, and roentgenographic skeletal survey. Clinical staging according to Durie and Salmon (9) was applied to all patients. Combination chemotherapy with the M-2 protocol was given every 5 weeks as follows: 0.5 mg/kg of BCNU iv on Day 1, 10 mg/kg of cyclophosphamide iv on Day 1, 0.25 mg/kg of melphalan orally on Days 1–4, 1 mg/kg of prednisone for 7 days and then tapered until Day 21, and 0.03 mg/kg of vincristine iv on Day 21 (6). If an objective response occurred, chemotherapy was continued until 12 cycles had been completed.

Dosage of the drugs was reduced when leukothrombocytopenia was present and/or in critically ill patients. Following the start of chemotherapy, paraprotein levels and bone marrow plasma cell rates were serially evaluated, usually every 2 months for the first 4 months and then every 3–4 months. Clinical response was evaluated in accordance with Myeloma Task Force criteria (2). The duration of response started when the paraprotein level fell to < 50% of the control value and was calculated until the paraprotein level climbed to twice its lowest level during remission. Patients were evaluated for response when at least two cycles of the M-2 protocol had been given. Survival was calculated from the start of combination chemotherapy by means of the life-table method (10). Responses and survival were calculated as of May 1982.

RESULTS

As of May 1981, 25 consecutive patients had entered this study. Thirteen were males, 12 were females, and the median age was 65 years (range, 37–79). The Karnofsky performance score was < 70 in 62% of the patients. Nineteen patients were classified as being in stage III, three were in stage II, and three were in stage I. However, all patients had symptomatic disease. Twelve patients had IgG myeloma (four κ type and eight λ type), four had IgA myeloma (three κ type and one λ type), one had IgD myeloma of the λ type, and eight had micromolecular myeloma with measurable κ (four patients) and λ (four patients) light chains.

The median follow-up time was 23 months (range, 3–75). Four patients were not evaluable for response because they did not receive at least two cycles of the M-2 protocol. In 17 of the 21 (80.9%) evaluable patients, an objective response was achieved. Patients with stage III or stage I–II disease responded equally well (80% versus 81.2%, respectively). There was no difference in response with respect to the type of myeloma proteins secreted. The median time from the first cycle of M-2 to an objective response was 2 months. The median duration of remission is 18 + months (range, 3–69 +).

All 25 patients are evaluable for survival. The actuarial 4-year survival curve is shown in figure 1. The median duration of survival was 42 months. In the first 15 months of follow-up, 20% of the patients died.

Toxicity was not negligible. During the entire period of the treatment, leukopenia and thrombocytopenia were encountered in 20 (80%) and four (16%) patients, respectively. Leukopenia was mild (2000–3000 cells/mm^3) in two patients, moderate (1000–2000 cells/mm^3) in ten patients, and severe (< 1000 cells/mm^3) in eight patients, whereas thrombocytopenia was moderate (50,000–100,000 platelets/mm^3) in three patients and severe (< 50,000 platelets/mm^3) in one. Treatment-related death occurred in the patient with severe thrombocytopenia. Among patients with severe leukopenia, the treatment was stopped in one, and another had pneumococcal meningitis.

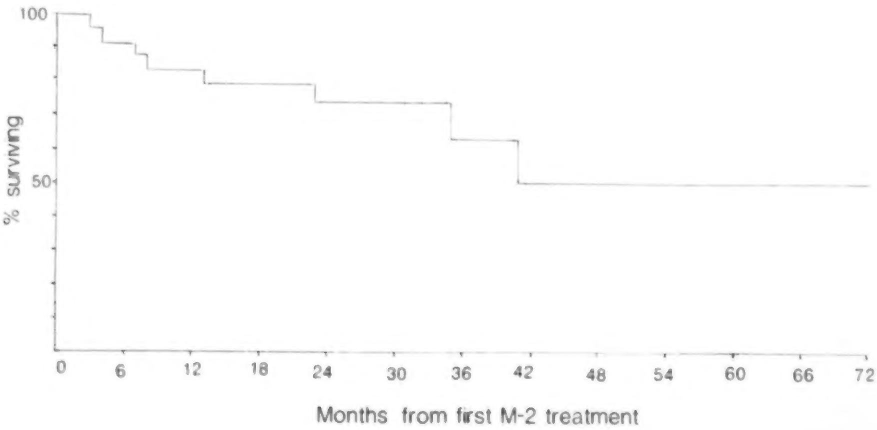

FIGURE 1.—Survival of patients from first M-2. The Nos. of patients at risk at Months 0, 6, 12, 18, 24, 30, 36, and 42 are 25, 23, 21, 14, 9, 6, 3, and 1, respectively.

DISCUSSION

Our results obtained in a group of 25 consecutive patients with previously untreated and symptomatic disease seem to confirm the validity of the M-2 protocol in MM. In particular, the high remission rate, the median time to response, and the length of survival agree with the Memorial Hospital data (1). However, the drop in early mortality in the first 15 months from diagnosis, as reported by the Memorial Hospital group (1), was not supported by our analysis from the start of therapy (5% versus 20%, respectively). Moreover, the grade of toxicity we observed seems higher, although careful reductions of drug dosages have been made, when necessary. In our study, survival was calculated from the beginning of the M-2 protocol treatment, instead of from diagnosis, as done at Memorial Hospital. In the latter way, it could be that patients with asymptomatic MM were not treated for many months, thus improving the early part of a survival curve measured from diagnosis. Moreover, our group of patients have been treated in a large general hospital, not at a referral center such as Memorial Hospital, where subsequent possible selection of patients may have occurred. Nevertheless, the conclusions of the Memorial Hospital group that the M-2 protocol is a more effective treatment than single therapy or melphalan-prednisone therapy on the basis of historical comparisons (1) may be supported even by our results. However, a randomized study comparing melphalan, prednisone, cyclophosphamide, and BCNU (four of the drugs present in the M-2 protocol) with melphalan plus prednisone did not show any significant difference (8). The M-2 protocol includes vincristine, a drug able to add 9 months to the responses obtained with alkylating agent combinations (11) and to induce a further reduction of tumor cells once the labeling index has increased (12).

In conclusion, the Eastern Cooperative Oncology Group randomized study comparing vincristine, BCNU, melphalan, cyclophosphamide, and prednisone with melphalan plus prednisone could clarify the real impact of the M-2 protocol on the treatment of MM.

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BRIEF REPORTS

Results of a Randomized Double-Blind Crossover Trial of Scopolamine Versus Placebo Administered by Transdermal Patch for the Control of Cisplatin-Induced Emesis[1]

Dan L. Longo,* Margaret Wesley, Diane Howser, Susan M. Hubbard, Tom Anderson, and Robert C. Young[2]

We tested the efficacy of scopolamine delivered by transdermal patch in preventing emesis induced by cisplatin as a single agent or in combination. The administration of scopolamine by transdermal patch resulted in acceptable side effects but was ineffective in controlling cisplatin-induced emesis. [Cancer Treat Rep 66:1975–1976, 1982]

Cisplatin is one of the most effective chemotherapeutic agents in the armamentarium of the clinical oncologist. Unfortunately, it is one of the most potent emesis-inducing agents as well. Many antiemetic regimens have been unsuccessful in controlling cisplatin-induced emesis. In the current study, we employed scopolamine in an effort to relieve the nausea and vomiting produced by cisplatin.

The belladonna alkaloid scopolamine, a muscarine antagonist, is attractive as a potential antiemetic because of its efficacy in preventing motion sickness (1). The drug is readily absorbed when administered orally or im, however, it produces a variety of side effects such as mouth dryness, blurred vision, tachycardia, anxiety, and behavioral changes through its parasympatholytic action. It is possible that some of these side effects are due to peak serum levels achieved when scopolamine is delivered orally or im. The Alza Corporation, Palo Alto, CA, developed an adhesive patch impregnated with scopolamine capable of gradually delivering the drug transdermally over a period of about 72 hours.[3] The transdermal patch maintains continuous serum levels of scopolamine in the therapeutic range for the prevention of motion sickness.[3] We compared scopolamine administered by transdermal patch to a placebo patch for the capacity to alleviate cisplatin-induced nausea and vomiting and the incidence of scopolamine-related side effects.

After obtaining informed consent, 45 patients (20 receiving cisplatin, cyclophosphamide, vinblastine, bleomycin, and dactinomycin for testicular cancer; 20 receiving cisplatin, 5-FU, cyclophosphamide, and hexamethylmelamine for ovarian cancer; and five receiving cisplatin for breast cancer) were randomized to receive scopolamine or placebo by patch on cycle 1 of treatment and automatically crossed over to the other agent on cycle 2. Patients were stratified according to their underlying malignancy and the chemotherapy they were to receive. Each patient placed a test patch behind each ear 12 hours preceding the iv administration of cisplatin and left the patches in place for 72 hours. Supplemental antiemetics were given on request beginning no sooner than 3 hours after administration of cisplatin. The antiemetic given was thiethylperazine at a dose of 10 mg orally or im every 4–6 hours as needed. Evaluation of drug efficacy and side effects was determined by review of subjective answers to a questionnaire completed by the patient (a copy of the questionnaire is available on request) and by observation of the patient for the presence or absence of vomiting and parasympatholytic physical signs. The questionnaire included assessment of the degree of nausea, the number of vomiting episodes, the number of antiemetic pills or suppositories taken, effects on appetite, the presence of side effects (including drowsiness, dry mouth, blurred vision, rapid heart beat, and dysphoria), and the subjective evaluation of the degree to which the nausea and vomiting interfered with normal living. The questionnaire covered the 72-hour period after treatment with cisplatin chemotherapy. In addition, the patient's vital signs and

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[1] Received Feb 1, 1982; revised Aug 20, 1982; accepted Sept 1, 1982.

[2] Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3] Shaw J. Data on file with the Alza Corporation, Palo Alto, CA.

**Reprint requests to:* Dan L. Longo, MD, Medicine Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 12N226, National Institutes of Health, Bethesda, MD 20205.

Table 1.—Scopolamine compared to placebo in preventing chemotherapy-induced emesis

Diagnosis	No. of evaluable patients (median age)	Treatment program	Median No. of vomiting episodes (range)	
			Scopolamine*	Placebo*
Ovarian cancer	15 (52 yrs)	Cyclophosphamide, 150 mg/m² orally on Days 1–15; hexamethylmelamine, 150 mg/m² orally on Days 1–15; 5-FU, 600 mg/m² iv on Days 1 and 8; and cisplatin, 30 mg/m² iv on Days 1 and 8	13 (1–134)	14 (2–65)
Testicular cancer	11 (23 yrs)	Cyclophosphamide, 1000 mg/m² iv on Day 1; vinblastine, 4 mg/m² iv on Day 1; dactinomycin, 1 mg/m² iv on Day 1; bleomycin, 15 units/m² iv on Day 1; cisplatin, 100 mg/m² iv on Day 7	15 (5–45)	11 (2–45)
Breast cancer	4 (48 yrs)	Cisplatin, 70 mg/m² iv every 21–28 days	8 (7–11)	12 (2–21)

*The order of administration was randomized and the test patches were always placed 12 hrs before the iv administration of cisplatin.

Table 2.—Side effects of transdermal patches

Side effects	No. of patients who experienced side effects with—			
	Scopolamine and placebo	Scopolamine only	Placebo only	Neither
Dry mouth	20 (54%)	11 (30%)	2 (5%)	4 (11%)
Blurred vision	5 (14%)	14 (38%)	0 (0%)	18 (49%)
Tachycardia	2 (5%)	7 (19%)	4 (11%)	24 (65%)
Dysphoria	4 (11%)	8 (22%)	4 (11%)	21 (57%)
Palpitations	4 (11%)	3 (8%)	2 (5%)	28 (76%)
Drowsiness	18 (49%)	4 (11%)	6 (16%)	9 (24%)
Behavioral changes	3 (8%)	2 (5%)	0 (0%)	32 (86%)
Fainting and dizziness	1 (3%)	2 (5%)	3 (8%)	31 (84%)

pupil response were measured every 4 hours while awake for the first 36 hours, and the number, times, and volume of emesis were recorded.

Thirty patients (67%) were evaluable to examine the question of antiemetic efficacy and 37 (82%) were evaluable to examine the side effects of the transdermal patch. The major reason patients were not evaluable was failure to complete the questionnaire or the use of qualitative answers such as "a lot" where quantitative data were requested. The statistical analysis was done by the techniques described by Koch (2) for nonparametric methods of looking at the two-period changeover design. There was no evidence for residual effects of one agent carrying over into the test period of the second agent, and the order in which the drug and placebo were given did not affect outcome. Using two-sided tests for significance, scopolamine was found not to be significantly more effective than placebo at preventing chemotherapy-induced emesis for the whole population or any subset of patients (table 1). However, two patients experienced dramatic reduction in the number of vomiting episodes with scopolamine as did two patients receiving placebo. There were no features that identified patients more likely to respond.

The side effects of the transdermal patches are listed in table 2. Most patients reported dry mouth and drowsiness whether they received scopolamine or placebo. Patients were more likely to have dry mouth on scopolamine (two-sided test; $P = 0.02$), but 61% of placebo-treated patients had dry mouth. Patients were more likely to have blurred vision on scopolamine (two-sided test; $P = 0.001$), but 13% of patients had blurred vision on placebo. There were no differences between scopolamine- and placebo-treated groups in the incidence of tachycardia, dysphoria, palpitations, drowsiness, behavioral changes, fainting, or dizziness.

The transdermal patch method of delivering scopolamine does appear to minimize unpleasant side effects. Unfortunately, scopolamine is of limited value in the treatment of cisplatin-induced nausea and vomiting. It is possible that scopolamine may be more useful against emesis induced by other chemotherapeutic agents. Certainly, the efficacy scopolamine has been shown to have in preventing motion sickness (1) and the reduced incidence of side effects it causes when delivered transdermally make it an attractive candidate for further testing.

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Salvage Chemotherapy for Non-Hodgkin's Lymphoma[1]

R. P. Ng, D. Todd,* and R. K. K. Khoo[2,3]

Nineteen patients with advanced non-Hodgkin's lymphoma resistant to previous chemotherapy were treated with combination chemotherapy consisting of lomustine (CCNU), cytarabine, and prednisone. Eight patients (42%) had complete remission and four (21%) had partial remission. The median survival time of the 19 patients was > 14 months. The eight patients who had complete remission were still alive as of May 1, 1982, with a median follow-up time of 632 days (range, 365–1247). We believe that this combination chemotherapy scheme warrants further investigation. [Cancer Treat Rep 66:1977–1979, 1982]

Unsuccessful initial chemotherapy for advanced non-Hodgkin's lymphoma is often associated with a poorer response to subsequent therapy (1). A review of the literature shows that the remission rates and survival times for patients who had had prior chemotherapy were generally lower than those for patients who had not received prior chemotherapy (2–4).

Most chemotherapy regimens consist of cyclophosphamide, vincristine, and prednisone, with the addition of doxorubicin, bleomycin, and/or methotrexate with leucovorin rescue (2–7). Because cytarabine (Ara-C) and lomustine (CCNU) can each produce a response rate of about 20%–30% in advanced non-Hodgkin's lymphoma (8) and because these drugs are not generally used in most of the initial combination chemotherapy schemes, we have investigated the use of the combination of CCNU, Ara-C, and prednisone in patients with non-Hodgkin's lymphoma who had either failed to achieve complete remission (CR) following initial chemotherapy or had had a relapse after initial response. This is a preliminary report of the results.

PATIENTS AND METHODS

Nineteen patients who had stage III/IV non-Hodgkin's lymphoma and failed induction or had a relapse were started on the PAC regimen, which consisted of prednisone (80 mg/m^2/day orally for 5 days starting on Day 1); Ara-C (100 mg/m^2/day sc for 5 days starting on Day 1); and CCNU (40 mg/m^2 orally on Day 1). This regimen was administered every 4 weeks.

Chemotherapy was not restarted for an additional week in the presence of significant thrombocytopenia ($< 80{,}000$ cells/mm^3) and/or leukopenia (< 3000 cells/mm^3).

Patient characteristics, including details of disease, treatment, and sites of involvement, are shown in table 1. Except for patient 16, none of the patients had blast cells in the peripheral blood.

All biopsy specimens were classified according to the criteria of Rappaport (9), and performance status was classified according to the World Health Organization classification (10). CR is defined as the absence of all clinical and radiologic evidence of disease and, in those with bone marrow involvement, the absence of evidence of disease on bilateral iliac crest trephine biopsy. Partial remission (PR) is defined as a > 50% reduction in measurable tumor size, and in the cases where there was initial marrow involvement, the absence of clinical evidence of disease but with residual marrow infiltration. Patients who achieved CR were maintained on courses of PAC every 2 months for a maximum of six courses.

RESULTS

The patients' responses to chemotherapy are listed in table 1.

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[1] Received Mar 15, 1982; revised July 23, 1982; accepted Aug 5, 1982.

[2] Department of Medicine, University of Hong Kong (*R. P. Ng* and *D. Todd*), and Institute of Radiotherapy and Oncology (*R. K. K. Khoo*), Queen Mary Hospital, Hong Kong.

[3] We thank Dr. F. C. S. Ho, Department of Pathology, Hong Kong University, for her help.

**Reprint requests to:* Prof. D. Todd, Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong.

TABLE 1.—Patient characteristics*

Patient No.	Sex/ age in yrs	Stage	Histology	Performance status	Residual disease site at relapse	Previous treatment	Response to PAC	Survival in days from start of PAC
1	F/68	IV B	DLPD	2	Marrow	CHOP	PR	196
2	F/60	IV A	DLPD	0	Skin	CHOP	CR	456 +
3	M/38	IV B	DLPD	1	Marrow	CHOP	CR	826 +
4	M/52	IV B	DLPD	0	Skin	CHOP + RT	PR	160 +
5	M/52	IV B	DLPD	4	Marrow, pleura	CHOP, CPOB	NR	29
6	F/49	IV B	DLPD	1	Marrow	CHOP	PR	673 +
7	F/65	III B	DM	2	Lymph nodes	CHOP	CR	435 +
8	M/44	IV B	DM	3	Pleura	CHOP	NR	54
9	M/54	IV B	DM	1	Marrow	HOP	CR	690 +
10	F/46	IV B	NLPD	0	Marrow	CVP, CHOP	CR	365 +
11	F/51	III B	NLPD	1	Lymph nodes	CHOP, COPP	CR	768 +
12	F/51	IV A	DLWD	2	Marrow	CVP, CHOP	PR	329
13	F/61	IV B	Undif	4	Marrow, CNS	CHOP	NR	100
14	M/20	IV A	Undif	3	Marrow, skin	HOP, COPP	NR	115
15	F/44	IV B	DH	1	Marrow	CHOP	CR	573 +
16	M/23	IV B	DH	3	Marrow	CHOP	NR	81
17	M/68	IV B	DH	2	Liver, CNS	CHOP	NR	31
18	M/68	IV A	DH	3	Peritoneal seedlings	CHOP	NR	53
19	M/44	III B	DH	3	Lymph nodes	CHOP, CPOB	CR	1247 +

*DLPD = diffuse lymphocytic poorly differentiated; DM = diffuse mixed histiocytic/lymphocytic; NLPD = nodular lymphocytic poorly differentiated; DLWD = diffuse lymphocytic well-differentiated; Undif = undifferentiated; and DH = diffuse histiocytic. CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; RT = radiotherapy; CPOB = cyclophosphamide, prednisone, vincristine, and bleomycin; HOP = doxorubicin, vincristine, and prednisone; CVP = cyclophosphamide, vincristine, and prednisone; and COPP = cyclophosphamide, vincristine, procarbazine, and prednisone. NR = no response. + = still alive.

Of the 19 patients, eight (42%) achieved CR, four (21%) had PR, while seven (37%) showed no response. Of the seven who did not show any response to PAC, six were severely ill with advanced disease and were able to receive only one or two courses of chemotherapy, and all seven have died, with a median duration of survival of 54 days. The eight patients who had CR are still alive as of May 1, 1982, with a median follow-up time of 632 days (range, 365–1247). Only two patients (Nos. 9 and 15) had evidence of relapse. Using life-table analysis the 50% probability of survival for the 19 patients is > 14 months.

The major side effects were nausea, vomiting, and marrow suppression. The nausea and vomiting was made tolerable by administration of concomitant metoclopramide. A total of 96 courses of PAC have been administered to these 19 patients; 11 episodes of leukopenia < 2000 cells/mm^3 and six episodes of thrombocytopenia $< 100{,}000$ cells/mm^3 were recorded. There were five episodes of both leukopenia and thrombocytopenia. The blood count nadirs usually occurred in weeks 3–4, mostly after the third course of chemotherapy. There was no death due to drug toxicity.

DISCUSSION

Results of chemotherapy in patients with non-Hodgkin's lymphoma who had had previous chemotherapy have generally been poor (1–4), and the response rates and survival times in our study compare favorably with these previous reports. Because CCNU is given orally and Ara-C is given sc, the PAC regimen can be readily administered in the outpatient clinic. This implies that the quality of life of these patients on the whole is better than that of patients whose chemotherapy must be administered in the hospital and in a protective environment. The drugs in the PAC regimen are also relatively inexpensive.

The dose of CCNU used in this study was smaller than the conventional dose reported in the literature, based on our experience with cytotoxic drugs in Chinese patients (unpublished observation) and based on the administration of CCNU once every 3–4 weeks rather than the usual 6 weeks.

The effectiveness, ease of administration, and relatively low cost of PAC make this an attractive regimen, and we believe that it warrants further trial as a salvage method.

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BRIEF REPORTS

Ip Immunotherapy and Chemotherapy in Advanced Epithelial Ovarian Cancer[1,2]

Enrique Hernandez,* Neil B. Rosenshein, Jose Pino y Torres, Jose Villar, Francis C. Grumbine, David S. Ettinger, Michael B. Dillon, Jerry L. Klein, and Stanley E. Order[3]

Nine patients with advanced epithelial ovarian cancer, classified as either stage III with macrometastatic residual disease or stage IV, received combination chemotherapy in conjunction with ip rabbit-derived human ovarian antitumor serum (HOATS). The HOATS regimen consisted of the ip instillation of 100 ml of ovarian antitumor serum on Days 1 and 3 of the first chemotherapy cycle. Side effects attributable to HOATS were low-grade fever in three patients and diffuse skin rash in two. The 1-year cumulative survival was 87%, with a clinical response rate of 80%. No significant toxic reactions to HOATS have been observed. Continuation of the present study seems justified. [Cancer Treat Rep 66:1981–1982, 1982]

Advanced ovarian carcinoma (stages III and IV) is almost uniformly fatal, with a 5-year survival rate of < 10% (1). Therapeutic management typically consists of tumor-reductive surgery, followed by either chemotherapy, radiotherapy, or chemotherapy plus nonspecific immunotherapy (1–8). Systemic chemotherapy is currently the preferred treatment modality for patients with advanced disease, as the results with radiotherapy in patients with large residual tumors have been discouraging (9,10).

A new approach to the management of this disease is the administration of ip immunotherapy in combination with multiagent chemotherapy. The use of these two modalities seeks to take advantage of the improved response observed in an ovarian cancer model when chemotherapy was combined with tumor-specific immunotherapy (11). Preliminary data on the use of this unique technique are presented.

METHODS

The study population consisted of patients with stage III or IV epithelial ovarian adenocarcinoma staged according to the International Federation of Obstetrics and Gynecology classification. Stage III was subdivided into micrometastatic (residual tumor nodules measured < 5 mm and covered < 50% of the peritoneal cavity) and macrometastatic (residual tumor nodules measured ≥ 5 mm or covered ≥ 50% of the peritoneal cavity). This report deals only with patients having stage III macrometastatic or stage IV disease, with histology of serous, mucinous, or undifferentiated adenocarcinomas and with no prior history of receiving chemotherapy or radiation. All patients were fully active at the initiation of therapy.

The chemotherapy regimen, given on a 28-day cycle, consisted of 150 mg/m^2 of hexamethylmelamine given orally on Days 1–14 and 500 mg/m^2 of cyclophosphamide given iv on Day 1, with 40 mg/m^2 of doxorubicin given iv on Day 1 alternating with 50 mg/m^2 of cisplatin given iv on Day 1 every other cycle. Prior to the initiation of chemotherapy, all patients had adequate hematologic, renal, and liver functions.

Human ovarian antitumor serum (HOATS) is prepared from patients with ovarian adenocarcinoma of mucinous, serous, or undifferentiated type. The technique of HOATS preparation has been previously described (12). The treatment regimen consisted of initial skin and eye testing followed by the ip instillation of 100 ml of HOATS on Days 1 and 3 of the first chemotherapy cycle. This was done by placing an intraabdominal catheter, demonstrating free peritoneal flow with iodinated contrast material, and then infusing the HOATS.

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[1] Received Mar 18, 1982; revised July 16, 1982; accepted Aug 5, 1982.

[2] Supported in part by Public Health Service grants CA-25905 and CA-17970 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a Clinical Fellow Award to Dr. Enrique Hernandez from the American Cancer Society.

[3] Divisions of Gynecologic Oncology (*E. Hernandez, N. B. Rosenshein, J. Villar, F. C. Grumbine,* and *M. B. Dillon*), Radiation Oncology (*J. Pino y Torres, J. L. Klein,* and *S. E. Order*), and Medical Oncology (*D. S. Ettinger*), The Johns Hopkins Medical Institutions, Baltimore, MD.

**Reprint requests to:* Enrique Hernandez, MD, Division of Gynecologic Oncology, The Johns Hopkins Hospital, 600 N Wolfe St, Baltimore, MD 21205.

Informed consent was obtained and the patients were then stratified by performance status, tumor grade, and histology, and randomized to receive or not receive HOATS in conjunction with the chemotherapy.

RESULTS

From January 1980 to July 1981, nine patients who met the above criteria were treated with HOATS and chemotherapy. Initially, four patients received HOATS as part of the pilot program. Five patients received HOATS as part of the ongoing prospective randomized study. Clinically evaluable disease was present in five patients. A clinical response was documented in four patients (complete response in 40%, partial response in 40%). The 1-year cumulative survival for these patients was 87%. The observed side effects attributable to HOATS were low-grade fever in three patients and diffuse skin rash in two. No significant increase in bone marrow toxicity was noted in this group of patients.

DISCUSSION

In the past three decades, there has been no significant improvement in the survival of patients with ovarian cancer. If we are to improve the survival of these patients, unique forms of therapy need to be explored. Our present HOATS protocol is such an effort.

It has been demonstrated in an ovarian cancer model that host sensitization by antiserum was therapeutic if host macrophages were available (13–15). In addition, an improved response was noted in this model when chemotherapy was combined with antiserum (11). The combination of HOATS and chemotherapy seeks to take advantage of these observations.

HOATS consists of polyclonal antibodies produced against multiple antigenic moieties found on the epithelial ovarian tumors. These antigenic moieties have also been identified in normal tissues (16). The administration of HOATS ip enables the antibodies to preferentially attach to the intraperitoneal cancer rather than to the similar antigenic moieties found in the circulation. As HOATS is a foreign protein, the antibody-antigen complex formed stimulates the host's immune system, activating intraperitoneal macrophages.

We have encountered no significant toxic reactions to HOATS, and patient survival has not been compromised. It seems justified to continue with the stratified randomized study.

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Autologous Bone Marrow Transplantation in Acute Myeloid Leukemia in Relapse or in Complete Remission[1]

P. Herve,[2,*] A. Rozenbaum,[3] E. Plouvier,[3] M. Flesch,[3] J. Y. Cahn,[3] A. Farradji,[4] J. P. Bergerat,[4] J. C. Adjizian,[5] and B. Pignon[5,6]

We report ten cases of acute myeloid leukemia treated by intensive therapy followed by autologous bone marrow transplantation. Seven patients were in first relapse, and three were in complete remission. The conditioning regimen consisted of either chemotherapy alone (6-thioguanine, cytarabine, lomustine, and cyclophosphamide [TACC; eight patients]) or cyclophosphamide and total-body irradiation (two patients). All the patients in first relapse achieved complete remission (CR). The median remission duration was 9.9 months (range, 5–14), and the median survival was 14.4 months (range, 9–23.8). Of the three patients autografted during CR, one relapsed at Month 5 and two others remain in CR and are well at 18+ and 18.3+ months. High-dose chemotherapy followed by autologous bone marrow transplantation seems to be a valuable treatment for acute myeloid leukemia, if it is used immediately after the CR to consolidate the remission. [Cancer Treat Rep 66:1983–1985, 1982]

Autologous bone marrow transplantation (ABMT) following high-dose chemotherapy with or without total-body irradiation (TBI) represents a new approach to the treatment of patients with acute myeloid leukemia (AML) without HLA-identical donors, either when conventional therapy has failed or to consolidate and hopefully to prolong a complete remission (CR). In this paper, we report the antitumor effect of high-dose chemotherapy in ten patients with AML. They were entered into our regional therapeutic protocol between April 1979 and June 1981.

PATIENTS AND METHODS

Patients.—The group consisted of ten patients (eight adults and two children) with AML. Subtypes of AML, according to the FAB classification (1), were as follows: M_2 (seven patients), M_1 (two), and M_6 (one). The median age was 28.2 years (range, 2–45). After diagnosis, all patients received extensive induction and consolidation therapy, and four of seven patients grafted in relapse had received maintenance therapy (table 1). Nine patients had marrow collection and storage during the first CR, and one during the second CR. Seven patients had high-dose chemotherapy and ABMT during the first relapse, and three others, during the first remission.

Bone marrow processing and cryopreservation.—These methods have already been described (1–3). Briefly, the bone marrow was enriched with a blood separator (Haemonetics model 30). DMSO in plasma was added to a final 10% concentration. The marrow cells were frozen at a slow rate in a freezer with an electronic programmer and stored in liquid nitrogen. After thawing, a stepwise dilution was employed followed by a centrifugation allowing the removal of supernatant with hemoglobin and DMSO. The marrow cells were infused immediately.

Conditioning of patient for marrow transplantation.—The conditioning regimen consisted either of a high-dose chemotherapy combination containing 6-thioguanine, cytarabine, lomustine, and cyclophosphamide (TACC; eight patients) (4) or of cyclophosphamide plus 10-Gy TBI (two patients). We modified the TACC regimen by increasing the dose of 6-thioguanine from 200 to 400 mg/m^2 daily over 4 days, of cytarabine from 200 to 400 mg/m^2 daily over 4 days, and of lomustine

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[1] Received Mar 22, 1982; revised July 13, 1982; accepted Aug 5, 1982.

[2] Centre Regional de Transfusion Sanguine, Besançon, France.

[3] Cliniques d'Hématologie, Hôpital Saint-Jacques, Besançon, France.

[4] Clinique d'Hématologie, Hôpital de Hautepierre, Strasbourg, France.

[5] Laboratoire d'Hématologie, Service de Médecine, Hôpital Robert Debre, Reims, France.

[6] We thank Miss E. Tamayo and Mr. A. Jeanroy for excellent technical assistance, and Miss E. Genevois for excellent secretarial help.

[*] *Reprint requests to:* P. Herve, MD, Centre Regional de Transfusion Sanguine, 15, rue Megevand, BP 1181, F 25003 Besançon Cedex, France.

TABLE 1.—Clinical characteristics of patients, response of leukemia, and follow-up after ABMT

Patient No.	FAB classification	Age (yrs) and sex	Time from onset of leukemia (mos)	Past drug therapy*	Maintenance treatment	Clinical status at time of ABMT	Conditioning regimen†	Response	Outcome	Survival (mos)
1	M$_2$	45 M	16	CA, DNR, MTX, 6MP	Yes	1st relapse	TACC	CR	Relapse (13th mo)	23.8
2	M$_2$	35 M	3	CA, DNR, VCR, 6TG	No	1st relapse	Cy + TBI	CR	Relapse (5th mo)	7.3
3	M$_2$	22 F	32	CA, DNR, 6TG, 6MP	Yes	1st relapse	TACC	CR	Relapse (12th mo)	14
4	M$_1$	25 M	9	CA, DNR, 6TG, 6MP	Yes	1st relapse	TACC	CR	Relapse (13th mo)	15.8
5	M$_2$	17 F	6	CA, DNR, 6TG	No	1st relapse	Cy + TBI	CR	Relapse (5th mo)	9
6	M$_2$	43 F	8	CA, DNR, 6TG	No	1st relapse	TACC	CR	Relapse (6.5th mo)	16
7	M$_2$	39 F	27	CA, DNR, 6TG	Yes	1st relapse	TACC	CR	Relapse (14th mo)	18+
8	M$_2$	44 M	6	CA, DNR, 6TG	No	1st CR	TACC	—	CR	18.3+
9	M$_1$	10 F	4	CA, DNR, 6TG	No	1st CR	TACC	—	CR	18+
10	M$_2$	2 M	6	VCR, CA, DNR, 6TG, Pred	No	2nd CR	TACC	—	Relapse (5th mo)	12+

*CA = cytarabine, DNR = daunorubicin, MTX = methotrexate, 6MP = 6-mercaptopurine, 6TG = 6-thioguanine, VCR = vincristine, and Pred = prednisone.

†Cy = cyclophosphamide.

from 200 to 400 mg/m^2 over 1 day. Cyclophosphamide was given daily at the same dose of 45 mg/kg over 4 days (5).

RESULTS

Harvesting Data

In the ten patients, the median net volume of aspirate was 898 ml (range, 375–1250). The mean yield of nucleated cells was 1.16×10^{10} (range, 0.6–2.4). After concentration with a blood cell separator, the pool of buffy coat was only 20% of the original volume and contained 57.6% ± 15.54% (mean ± SD) of the total cells; 79% ± 9.2% (mean ± SD) of the CFU-GM were recovered in the final concentrate. The average total CFU-GM cryopreserved was 2.75×10^6 (range, 0.2–8.8).

Toxicity of the Conditioning Regimen

Two severe cardiac complications occurred during initial treatment: one patient who died within 6 days of ABMT is not included in this study; the other patient (No. 7) developed congestive heart failure within 10 days of ABMT, which responded to treatment. Gastrointestinal symptoms were observed in almost every patient (nausea, vomiting, and diarrhea). Stomatitis was seen in two patients. No side effects were observed after transfusion of thawed marrow cells, except for three patients who developed fever 1 hour after reinfusion.

Kinetics of Hematopoiesis Recovery

Six patients achieved granulocyte counts $> 0.5 \times 10^9$/L between Days 14 and 20. In three patients (Nos. 2, 5, and 6), hematopoietic recovery was delayed. Granulocyte counts $> 0.5 \times 10^9$/L were reached between Days 35 and 45, and platelet counts $> 50 \times 10^9$/L were reached between Days 40 and 80. Two of these patients had received a low number of nucleated cells (0.98 and 1.2×10^8/kg). For patients 8 and 9, grafted in CR, we observed a very delayed platelet recovery, though the number of nucleated cells infused was considered adequate.

Antileukemia Effects (Table 1)

All seven patients treated in first relapse with TACC or cyclophosphamide + TBI achieved a second CR. The median remission duration was 9.9 months (range, 5–14). The addition of TBI to the chemotherapy did not improve the duration of second CR for patients 2 and 5. The median survival was 14.4 months (range, 9–23.8). Only one patient (No. 7) is still alive (18+ months). The second CR was > 10 months in four of the seven patients. For five of the seven patients, survival was ≥ 1 year. The quality of life after ABMT was good, and most patients treated with chemotherapy alone and achieving hematopoietic recovery stayed in the hospital for a short time (30 days on average).

Of the three patients treated during CR, two are in CR and well (18+ and 18.3+ months), without any maintenance treatment. Patient 10 relapsed at the fifth month but achieved a third CR after high-dose melphalan (200 mg/m^2) followed by rescue with thawed bone marrow collected 2 months before. He has remained in his third CR for 7 months.

DISCUSSION

Case reports show that high-dose cytoreductive regimens followed by ABMT can induce CR in relapsed acute leukemia. The TACC regimen appeared effective when employed early in relapse. The patients were not selected; however, in all cases but one (patient 10), the bone marrow was collected early in the course of the first remission.

For AML, the incidence of CR has improved during recent years, but a very small proportion of the patients who achieve CR can expect to be cured. Duration of the first CR in AML still remains short.

Intensive cytoreductive treatment followed by ABMT can be used in two different situations in AML: either in relapse (6) or immediately after CR is reached, to consolidate and possibly increase duration of remission. After ABMT, maintenance chemotherapy can be avoided and the quality of life is better.

For the AML patients grafted during CR, a comparative study of the antileukemic effect of two different conditioning regimens, such as chemotherapy alone versus TBI plus cyclophosphamide, must be evaluated. However, the toxicity of the conditioning regimen must be taken into account before a patient in CR is entered into this protocol. We have had a patient treated during CR who died before marrow grafting because of cardiac failure. A complete evaluation of risks should be made and the patient should be informed of them before agreement to the procedure. More patients treated earlier in CR are needed to compare this treatment modality, in terms of survival, with conventional chemotherapy.

One fundamental problem remains: the bone marrow collected from leukemic patients during CR for autotransplantation contains residual malignant cells. Various approaches are proposed to remove leukemic cells. The bone marrow may be treated with cytotoxic drugs in vitro just before the cryopreservation step. Sharkis et al (7) have proposed the use of 4-hydroperoxycyclophosphamide, and we have started an ABMT protocol using this drug in vitro.

In conclusion, marrow viability after thawing and hematopoietic reconstitution even after TBI have been established, though in some cases the full reconstitution is delayed. However, several unresolved problems remain: (*a*) the effect of freezing on the megakaryocyte stem cell (platelet recovery is often delayed compared to granulocyte recovery); (*b*) the control of the nonmedullary toxic effects of intensive chemotherapy; (*c*) the antileukemic effect of chemotherapy alone versus chemotherapy plus TBI; and (*d*) the means to remove the residual leukemic cells from harvested bone marrow.

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BRIEF REPORTS

Multiple Squamous Cell Carcinoma Treated With Intra-arterial Cisplatin in a Patient With Rheumatoid Arthritis[1]

Jaffer A. Ajani* **and Michael A. Burgess**[2]

A 64-year-old woman, who had had rheumatoid arthritis controlled for many years by prednisone, developed multiple biopsy-proven squamous cell carcinoma of the skin extending from the left elbow to the hand. She was treated with intra-arterial cisplatin (90 mg/m^2) every 4 weeks. After four treatments, she achieved a complete clinical remission that was maintained (8+ months) until she died of an unrelated cause. We believe that arterial infusion of cisplatin as treatment for advanced regional squamous cell carcinoma of the skin should be pursued further as part of a multimodal approach and as definitive therapy. [Cancer Treat Rep 66:1987–1989, 1982]

Squamous cell carcinoma (SCC) is a common tumor, most frequently found on surfaces exposed to ultraviolet radiation. Recent evidence suggests that patients with immunologic defects have a higher than expected incidence of SCC of the skin arising de novo (1–7). Such tumors are not infrequently multiple and may have a more malignant behavior than those arising from preexisting skin lesions (1,8). We describe here a patient with rheumatoid arthritis controlled over many years with steroids, who developed multiple SCC and was successfully treated with intra-arterial infusions of cisplatin.

CASE REPORT

A 64-year-old woman who had had severe rheumatoid arthritis for 39 years was referred to The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute at Houston for treatment of multiple SCC of the skin of the left upper extremity in June 1980. SCC had previously been excised from her left and right ankle regions in 1975 and from her left forearm in May 1980. Within 1 month of the latter surgery, multiple new tumors appeared over the left upper extremity.

The patient's rheumatoid arthritis had been well-controlled for many years by administering prednisone (20 mg/day). Examination revealed multiple joint deformities consistent with the diagnosis of rheumatoid arthritis. Extending from the elbow to the hand, there were approximately ten hard, nontender cutaneous nodules, each measuring $<$ 1 cm in diameter (fig 1A); in June 1980, biopsy was performed on four of the nodules, confirming the diagnosis of SCC. There was no lymphadenopathy in the axillary, cervical, or inguinal regions. The chest X-ray and liver chemistry evaluations did not reveal any evidence to suggest metastatic tumor.

In July 1980, intra-arterial cisplatin (90 mg/m^2) was infused through a catheter in the left brachial artery. A French arterial catheter (size 6.5) was advanced from the femoral artery to the brachial artery for infusion. Half-strength normal saline solution was used for prehydration and posthydration. Cisplatin was mixed in 500 ml of one-half normal saline and was infused over 2 hours. Before cisplatin infusion, 50 ml of 20% mannitol solution was given iv, and during cisplatin infusion, 100 ml of mannitol solution was given iv. Two weeks after the first treatment, the patient was admitted to the hospital with a diagnosis of acute myocardial infarction, from which she recovered uneventfully. Six weeks later the intra-arterial chemotherapy was resumed, every 4 weeks. All tumors regressed. After four treatments the patient was in complete remission (fig 1B). One further treatment was given in December 1980 to consolidate the excellent response. The patient did not develop any

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[1] Received June 9, 1982; revised Aug 12, 1982; accepted Aug 19, 1982.

[2] Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Jaffer A. Ajani, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

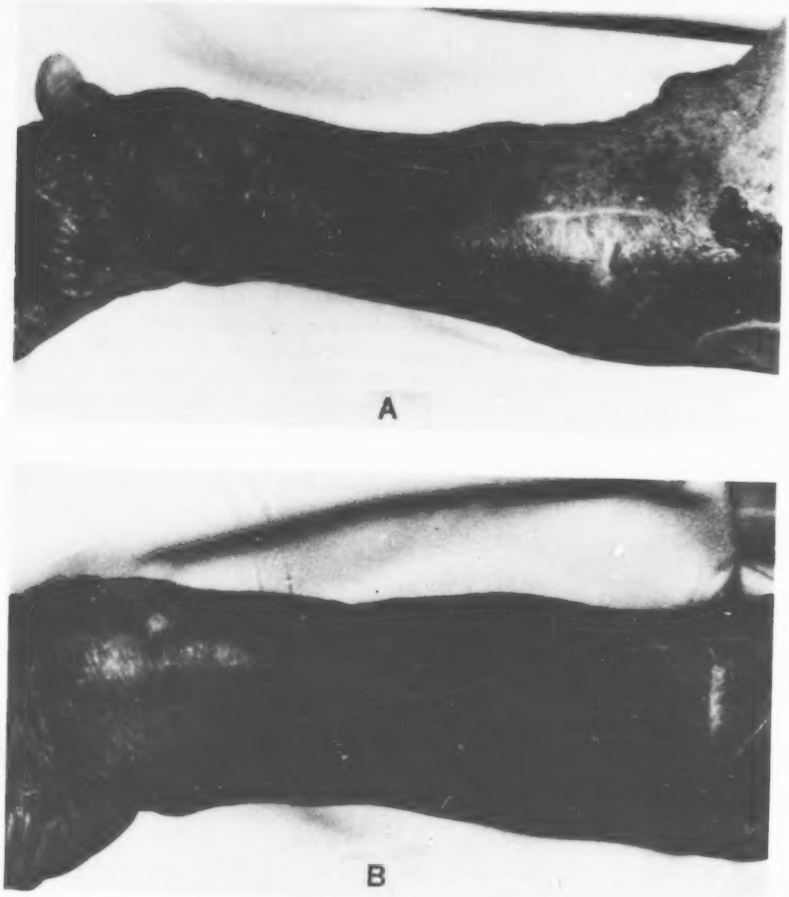

FIGURE 1.—64-yr-old woman treated for rheumatoid arthritis with prednisone developed SCC extending from left elbow to hand (A). After 4 intra-arterial treatments with cisplatin she had complete remission (B).

local or systemic toxic effects as a result of intra-arterial cisplatin.

On July 31, 1981, the patient was admitted to a local hospital, acutely ill and reporting low back pain. Clinically, there was evidence of gas gangrene involving the upper thigh. She developed cardiopulmonary arrest within hours of hospitalization and could not be resuscitated. Autopsy revealed an unsuspected carcinoma of the transverse colon but no evidence of residual SCC of the left upper extremity or anywhere else in the body.

DISCUSSION

Evidence suggests that, in this patient, the development of multiple SCC was related to long-term immunosuppressive therapy rather than to the underlying rheumatic disorder. For patients with extensive SCC, in whom the traditional therapeutic approaches of surgical excision and radiation therapy are not applicable, arterial infusions of chemotherapeutic agents including bleomycin, methotrexate, dactinomycin, vincristine, vinblastine, 5-FU, and hydroxyurea have been used with varying degrees of success (9).

The choice of cisplatin for this patient resulted from our experience with infusing this drug by the arterial route (10,11) and from its established activity against SCC when given systemically (12,13). The complete clinical remission in this patient for 8 months suggests that infusion of cisplatin as treatment for advanced regional SCC should be pursued further, both as a definitive therapy and as part of a multimodal approach. Successful preoperative regional chemotherapy may permit less radical surgical resection (amputation) in some patients.

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High-Dose AMSA and Autologous Bone Marrow Transplantation in Relapsed Acute Leukemia[1]

Axel R. Zander,* Lijda Vellekoop, Karel A. Dicke, Michael J. Keating, Kenneth B. McCredie, Sewa S. Legha, Gary Spitzer, Dharmvir Verma, and Gerald P. Bodey[2]

AMSA is a recently developed antitumor agent that has undergone clinical studies in solid tumors and leukemias (1–5). Since its dose-limiting toxic effect is myelosuppression, we undertook a study giving AMSA in high doses (800 mg/m^2) divided in four daily doses to patients with relapsed leukemia, followed by infusion of autologous bone marrow that had been stored during remission.

Eleven patients (median age, 27 years; range, 19–54) received a total of 13 high-dose AMSA treatments with bone marrow rescue. All patients except two had failed reinduction attempted before entering this program (table 1). The performance status ranged from 1 to 3 on the Zubrod scale. A median of 1×10^8 (range, 0.4–1.4×10^8) nucleated bone marrow cells/kg of body weight were infused. The viability of the bone marrow was checked with the in vitro culture of granulocyte macrophage precursors (CFC-GM). A median of 44,720 CFC-GM were infused (range, 3400–152,000). Five patients died during the treatment course with AMSA and bone marrow rescue. Three of the five patients had systemic *Candida* infections, which did not respond to amphotericin B, and died within 3 weeks after transplantation. Two patients died after a prolonged period of bone marrow aplasia (37 and 60 days) without evidence of bone marrow engraftment. In three patients leukemia occurred after an initial period of aplasia. Two of these patients received further investigational treatment. Three patients had responses: one patient (acute myelocytic leukemia) had hematologic improvement of 4 months' duration, one patient (acute lymphocytic leukemia) had a partial remission after the first course and progressing disease following recovery from the second course, and one patient (acute myelocytic leukemia) had a complete remission of 4 months' duration.

Major toxic effects consisted of fever in all 13 courses (11 patients). Systemic fungus infections were documented in three courses, and bacterial infections were documented in four. Nausea and vomiting occurred during all 13 courses, and severe stomatitis occurred in six. Transient liver function abnormalities were associated with systemic infections in six courses. Renal function abnormalities occurred in two patients, subsequent to treatment for systemic fungus infection. No cardiac side effects were noted.

The overall response rate was disappointing, with one complete remission among 11 treated patients. However, only two patients received AMSA as the initial treatment for the first relapse. The low response rate can be explained by extensive previous therapy and corresponds with other observations made with AMSA treatment in refractory leukemia (3). It appears that the infusion of cryopreserved autologous bone marrow was not able to modify the infectious complications of myelosuppression expected in this high-risk patient population. High-dose chemotherapy with autologous bone marrow rescue should be undertaken early in the course of treatment in patients with malignancies sensitive to chemotherapy, when an optimal response with minimal side effects can be expected.

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[1] Received Aug 14, 1981; revised July 12, 1982; accepted Aug 30, 1982.

[2] Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

**Reprint requests to:* Axel R. Zander, MD, Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Previous treatments and response to high-dose AMSA and autologous bone marrow rescue

Patient No./age (yrs)	Diagnosis*	Previous treatment†	Duration of treatment (mos)	AMSA treatment (mg)	Response	Outcome
1/25	AML	AdOAP → CR; AIM → F, POMP → F; CBV + ABMT → PR	34	800	Aplasia	Died on Day 37 of liver failure
2/28	AML	AdOAP → CR; Pip TBI + ABMT → CR	24	800	Failure	Subsequent chemotherapy
3/28	AMML	AdOAP → CR; ROAP → CR; CBV + ABMT → CR	49	800	Aplasia	Died on Day 19 of fungemia
4/25	ALL	AdOAP → CR; CROP → CR; POMP → F	35	800	Failure	Died on Day 15 of fungemia
5/30	APL	AdOAP → CR; CROP → CR; ara-C + 6-thioguanine → F; AdOAP → CR	24	800	Aplasia	Died on Day 10 of fungemia
6/25	AML	AdOAP → CR	23	800	Aplasia	Died on Day 60 of liver failure
7/40	ALL	AdOAP → CR; OAP	61	800	Partial response	Subsequent chemotherapy
8/22	AML	AdOAP → CR; OAP → CR	18	800 × 2	Failure	Subsequent chemotherapy
9/27	ALL	CHOP-Bleo → CR; AIM → CR; CBV + ABMT → F	32	800 × 2	Failure	Refused further chemotherapy
10/19	AML	AdOAP → CR	18	800	Complete response	Subsequent chemotherapy
11/54	AML	ROAP → CR; methyl-gag → F; AMSA → CR	22	800	Failure	No further chemotherapy

*AML = acute myelocytic leukemia; AMML = acute myelomonocytic leukemia; ALL = acute lymphocytic leukemia; APL = acute promyelocytic leukemia.

†AdOAP = doxorubicin, vincristine, cytarabine, and prednisone; CR = complete response; AIM = L-asparaginase, ifosfamide, and methotrexate; POMP = purinethol, vincristine, 6-mercaptopurine, and prednisone; CBV = high-dose cyclophosphamide, carmustine, and etoposide; ABMT = autologous bone marrow transplantation; PR = partial response; Pip TBI = piparazinedione and total-body irradiation; ROAP = zorubicin, vincristine, cytarabine, and prednisone; CROP = cyclophosphamide, zorubicin, vincristine, and prednisone; CHOP-bleo = cyclophosphamide, doxorubicin, vincristine, prednisone, and bleomycin.

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CLINICAL TRIALS SUMMARIES

m-AMSA in Disseminated Prostatic Carcinoma: A Phase II Study[1,2]

Anibal Drelichman,* David A. Decker, Muhyi Al-Sarraf, and C. B. Dhabuwala[3,4]

Prostatic carcinoma is a leading cause of morbidity and mortality from cancer in the elderly male population of the United States (1). Several chemotherapeutic agents have been explored for use in disseminated disease. Cyclophosphamide, 5-FU, cisplatin, and doxorubicin all demonstrated activity in phase II and III clinical trials (2–4). To determine the efficacy of *m*-AMSA, a new acridine derivative in the management of disseminated prostatic carcinoma, a phase II study was conducted at Wayne State University (5).

MATERIALS AND METHODS

Patients with histologically confirmed carcinoma of the prostate, regardless of previous treatment, were eligible. They were to receive no hormonal or cytotoxic therapy for > 4 weeks prior to entering the study. Before patients entered the study, extent of disease was documented by physical examination, bone scan, bone survey, pelvic computerized tomographic scan, and acid phosphatase. All patients were seen every 3 weeks, and lesions were measured. Pertinent tests were repeated as warranted during the course of therapy. Response was defined according to National Prostatic Cancer Project criteria (4).

Good-risk patients ($\leq$ 65 years of age, wbc count $\geq 4000/\text{mm}^3$, platelet count $\geq 100{,}000/\text{mm}^3$, bilirubin $\leq$ 1.5 mg/100 ml, prior radiation therapy to < 49% of functioning bone marrow, and/or no prior chemotherapy) received 120 mg/m^2 of *m*-AMSA every 3 weeks. Poor-risk patients (> 66 years of age, wbc count of $2500–3999/\text{mm}^3$, platelet count of $75{,}000–99{,}999/\text{mm}^3$, bilirubin of 1.6–3.0 mg/100 ml, radiation therapy to $\geq$ 50% of functioning bone marrow, and/or intolerance to prior chemotherapy) received 100 mg/m^2 of *m*-AMSA every 3 weeks.

RESULTS AND DISCUSSION

Twenty-six patients were entered in this study. Their characteristics, previous therapy, and response are presented in table 1. All patients were evaluable for toxic effects. Five patients were not evaluable for response; four died < 3 weeks from beginning of therapy, and one had repeated biopsies of the prostate that failed to prove the primary site. Twenty-five of 26 patients were poor-risk.

No complete or partial remissions were observed. Ten patients (47.6%) had stable disease, with a median survival of 48 weeks. Of the patients with stable disease, one had a < 50% decrease in size of pelvic nodes, two had improvement of myelophthisic anemia, and one had improvement in bone scan. The remaining 11 patients (52.2%) developed progressive disease, with a median survival of 14.4 weeks (table 1).

Toxicity was mainly hematologic. Two patients died from sepsis secondary to myelosuppression. Leukopenia was observed in 23 patients (88.5%); thrombocytopenia was observed in 12 (46.2%). Mild nausea and vomiting occurred in two patients. Two patients had mild alopecia, and one had skin hyperpigmentation.

We conclude that *m*-AMSA in this dose and schedule did not produce complete or partial remissions in this poor-risk population. The dose-limiting toxic effect, leukopenia, was severe in these patients, with two drug-related deaths.

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[1] Received Mar 16, 1982; revised Aug 3, 1982; accepted Aug 19, 1982.

[2] Supported in part by contract N01-CM-07404 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Departments of Oncology (*A. Drelichman, D. A. Decker,* and *M. Al-Sarraf*) and Urology (*C. B. Dhabuwala*), Wayne State University, School of Medicine, Detroit, MI.

[4] We thank Mary Beaker and Lynn McCloud for secretarial and data management support.

**Reprint requests to:* Anibal Drelichman, MD, Department of Oncology, Harper-Grace Hospitals, 3990 John R, Detroit, MI 48201.

TABLE 1.—*m*-AMSA in prostatic carcinoma: patient characteristics, response, and toxicity

	No. of patients (%)
Eligibility	
Total entered	26
Evaluable for toxic effects	26
Evaluable for response	21[*]
Age in yrs (*n* = 26)	
Mean: 67.3	
Range: 55–90	
Performance status (*n* = 26)	
Mean: 67.5	
Range: 40–90	
Predominant site of metastases (*n* = 26)	
Bone	23 (88.5)
Viscera	3 (11.5)
Previous therapy (*n* = 26)	
Orchiectomy	4 (14)
Additive hormonal	3 (12)
Orchiectomy and hormonal	19 (74)
Radiation[†]	16 (62)[‡]
Chemotherapy[†]	11 (43)[‡]
Response (*n* = 21)	
Complete/partial	0 (0)
Stable disease	10 (47.6)[§]
Progressive disease	11 (52.4)[¶]
Toxic effects (*n* = 26)	
Leukopenia	23 (88.5)
Mild/moderate	5 (19.3)
Severe/life-threatening	18 (69.2)
Thrombocytopenia	12 (46.2)
Mild/moderate	5 (19.2)
Severe/life-threatening	7 (26.9)
Nausea/vomiting	2 (7.0)
Skin hyperpigmentation	1 (3.0)
Alopecia	2 (7.0)

[*] 5 patients inevaluable for response because of early death (4 patients) and adenocarcinoma of unconfirmed primary site (1 patient).

[†] In addition to hormonal therapy.

[‡] % of all cases. Some patients had both.

[§] Median survival: 48 wks.

[¶] Median survival: 14.4 wks.

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CLINICAL TRIALS SUMMARIES

Phase II Trial of Mitoxantrone in Advanced Colorectal Cancer[1,2]

Eric M. Bonnem, Edith P. Mitchell, Paul V. Woolley,* Frederick P. Smith, John Neefe, Leroy Smith, and Philip S. Schein[3]

Mitoxantrone (DHAD) is an anthraquinone that has shown antitumor activity in several preclinical test systems. It exhibits less cardiotoxicity than doxorubicin in the rodent (1). Phase I studies of DHAD defined a tolerable dose of 8–12 mg/m^2 for phase II trials (2–5). Disease-oriented trials in acute leukemia (5) and breast carcinoma (6) have been reported. We report here a phase II study of DHAD in advanced colorectal carcinoma.

Between January and September 1981, 45 patients with advanced, histologically proven adenocarcinoma of the colon and rectum were treated with DHAD. Characteristics of this group are shown in table 1. Forty-one patients had received prior radiation therapy and/or chemotherapy, while four had not received prior therapy. Thirty-nine patients had been previously treated with some chemotherapy; all of these patients had received 5-FU, 47% had received nitrosoureas, 14% had received mitomycin, and several had received other investigational drugs. The median performance status was 1, and all patients had at least one site of objectively measurable disease. A partial response was defined as a $>$ 50% reduction in all sites of measurable disease, while a complete response was defined as complete disappearance of all measurable disease, with no appearance of disease elsewhere. The liver was considered to be a measurable lesion if it contained proven metastatic disease and was enlarged at least 5 cm below the costal margin. In 22 of the previously treated patients, the initial dose of DHAD was 8 mg/m^2 every 3 weeks; three of these patients had subsequent dose escalation from 8 to 10 mg/m^2 when no myelotoxicity had been demonstrated at the lower dose. Twenty-one patients were treated at a dose of 10 mg/m^2 later in the study when it became apparent that the 8-mg/m^2 dose was well-tolerated. Two patients were treated at a dose of 12 mg/m^2. The number of courses per patient is shown in table 2.

None of the 45 patients had a clinical response to DHAD treatment. The major toxic effect was dose-related myelosuppression (table 2), which was reversible in all cases. In those patients who received multiple doses, there was little variation in successive nadir counts, suggesting the absence of cumulative myelotoxicity. All of the patients who received repeat drug doses were able to receive the subsequent dose of DHAD on schedule. One patient who had received a dose of 10 mg/m^2 became febrile, with a wbc count of 1.2×10^3/mm^3; blood cultures grew *Enterobacter spp* that responded to gentamicin, and the patient recovered. This patient had been previously treated with 5-FU and semustine.

Nonhematologic toxicity was mild. Slight nausea and vomiting occurred in three patients. There were two episodes of gastrointestinal bleeding; one was thought to be secondary to a necrotic malignant ulcer. Both episodes were terminal events and neither was related to thrombocytopenia.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	45
Males	22
Females	23
Median age in yrs (range): 61 (31–78)	
Prior therapy	
None	4
Chemotherapy	23
Radiation	2
Chemotherapy + radiation	16
Median performance status (range): 1 (0–2)	

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[1] Received Apr 1, 1982; revised Aug 5, 1982; accepted Aug 19, 1982.

[2] Supported by contract N01-CM-97208 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Division of Medical Oncology, Department of Medicine, Georgetown University Hospital and Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, Washington, DC.

**Reprint requests to:* Paul V. Woolley, MD, Rm 414, Vincent T. Lombardi Cancer Research Center, 3800 Reservoir Rd, NW, Washington, DC 20007.

TABLE 2.—No. of courses of mitoxantrone and hematologic toxicity

	Dose (mg/m^2)		
	8	10	12
	No. of patients*		
No. of courses			
1	4	7	2
2	10	7	
3	5	5	
4	4	1	
	***Hematologic toxicity*†**		
Median wbc count nadir, × 10^{-3} cells/mm^3	3.3 (1.6–8.7)	3.4 (1.2–11.10)	1.5, 10.3‡
Median platelet count nadir, × 10^{-3} cells/mm^3	193 (80–494)	248 (105–416)	230, 234‡

*Each entry specifies No. of patients who received indicated No. of courses at each dose level.

†Values in parentheses = range.

‡Only 2 patients.

There were no cases of congestive heart failure; none of the patients had received anthracyclines. In 13 patients, disease progression was documented after one course, in 17 patients after two courses, in ten patients after three courses, and in five patients after four courses of DHAD (table 2).

In view of subsequent experience with DHAD, the doses used in this study were somewhat low. Previously treated patients were started at a dose of 8 mg/m^2 because at the time the trial was begun, there was information to suggest that the dose-response curve for toxicity at doses > 10 mg/m^2 was steep and that 14 mg/m^2 would be the maximal tolerated dose. While the 8- and 10-mg/m^2 doses were well-tolerated, they have been associated with toxic effects; 30% of the patients treated at a dose of 8 mg/m^2 and 40% of those treated at a dose of 10 mg/m^2 had wbc count nadirs $< 3 \times 10^3$/mm^3. No indication of drug activity that would encourage use of higher doses was seen. In summary, on the basis of these data, DHAD has no activity in advanced colon carcinoma in this dose range and schedule.

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CLINICAL TRIALS SUMMARIES

Phase II Trial of Bisantrene in Advanced Colorectal Cancer: A Cancer and Leukemia Group B Study[1,2]

Michael C. Perry,[3,*] Arlene A. Forastiere,[4] Frederick Richards, II,[5] Raymond B. Weiss,[6] and Dan Anbar[7]

Bisantrene is a synthetic anthracenedicarboxaldehyde hydrazone with antitumor activity in a variety of animal systems including colon line 26 (1). Its mechanism of action is unclear but in vitro studies indicate that bisantrene, which contains a planar electron-rich chromophore, may act as a DNA intercalating agent. In phase I studies the dose-limiting toxic reaction was reversible leukopenia, but nausea and vomiting, local irritation, phlebitis, chills, fever, hypotension, and anaphylactoid reactions were also observed (2). The activity of bisantrene against human tumors is unknown. Since there are few chemotherapeutic agents effective against advanced colorectal cancer, the Cancer and Leukemia Group B (CALGB) elected to evaluate the drug in patients with this disease.

MATERIALS AND METHODS

Eligibility criteria for this study included histologic confirmation of unresectable or metastatic colorectal carcinoma, measurable tumor, a CALGB performance score ≤ 3, the absence of cardiac disease, and informed consent. Patients were required to have a wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, hemoglobin ≥ 10 g/dl, BUN ≤ 25 mg/dl, creatinine ≤ 1.5 mg/dl, and bilirubin ≤ 1.5 mg/dl. At least 4 weeks had to have elapsed from major surgery or radiotherapy. Our goal was to study patients not previously exposed to chemotherapy. However, we did not exclude patients who had received adjuvant chemotherapy > 1 year prior to the study.

Based on phase I observations, 260 mg/m^2 of bisantrene was administered iv over at least 60 minutes every 3 weeks. One dose escalation to 280 mg/m^2 was permitted, and the protocol provided for dose reductions based on nadir blood cell counts and those obtained on the day of treatment. Minimum treatment was three cycles (9 weeks) unless there was objective evidence of disease progression.

RESULTS

Thirty-nine patients were entered in this study. Seven were ineligible because of prior chemotherapy in the year preceding entry, leaving 32 patients. There were 22 males and ten females, with a median age of 61.7 years (range, 33–75). All patients had a CALGB performance score ≤ 2. Twenty-one patients had been treated with surgery alone. Eight patients had received prior adjuvant chemotherapy, radiotherapy, or both. Sites of measurable disease included liver (20 patients), lung (11), abdominal masses (five), lymph nodes (four), subcutaneous masses (three), and bone (three). All patients received the first course of therapy, 26 received a second, 17 a third, four a fourth, and one a fifth course. There were 17 dose escalations and two reductions.

No patient had a partial or complete response. Nine patients were stable, 20 had progressive disease, and there were three early deaths, all tumor-related.

Hematologic toxicity was mild, with median nadir wbc counts of 4700/μl (range, 1100–6900) and platelet counts of 277,000/μl (range, 100,000–544,000). The most striking toxic reactions were fever, chills, and phlebitis (table 1). Following treatment, 12 patients (38%) experienced fever and chills, occasionally with hypotension. Nine patients (28%) reported burning at the iv

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[1] Received May 20, 1982; revised Aug 12, 1982; accepted Aug 19, 1982.

[2] Supported by Public Health Service grant CA-31946 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] University of Missouri Health Sciences Center, Columbia.

[4] Baltimore Cancer Research Center, University of Maryland.

[5] Bowman-Gray School of Medicine, Winston-Salem, NC.

[6] Walter Reed Army Medical Center, Washington, DC.

[7] Cancer and Leukemia Group B, Brookline, MA.

[*] *Reprint requests to:* Michael C. Perry, MD, Department of Medicine, N408, University of Missouri Health Sciences Center, Columbia, MO 65212.

TABLE 1.—Patient characteristics

	No. of patients
Eligible for study	32
Sex	
Male	22
Female	10
Median age (range) in yrs: 61.7 (33–75)	
Performance status	
0	14
1	9
2	9
Toxicity	
Nonhematologic	
None	11 (34%)
Fever/chills	12 (38%)
Hypotension	2 (6%)
Nausea/vomiting (≥ grade 2)	3 (9%)
Phlebitis	9 (28%)
Hematologic*	
Median nadir wbc count × 10^3 (range): 4.7 (1.1–6.9)	
Median nadir platelet count × 10^3 (range): 277 (100–544)	

*Data are based on 32 courses given at a dose of 260 mg/m^2.

site or developed thrombophlebitis following the infusion. Nausea and vomiting of at least grade 2 (moderate) severity were mentioned by only three patients (9%).

DISCUSSION

This phase II trial failed to identify any activity of bisantrene in patients with recurrent or metastatic colorectal cancer who had not received previous chemotherapy for metastatic disease. Side effects were fever, chills, and phlebitis. At this dose and schedule bisantrene is inactive in this disease.

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VAP-Cyclo: A Short Intensive Regimen of Chemotherapy for Advanced Breast Cancer

Combination chemotherapy will produce responses in 53%–84% of patients with metastatic breast cancer who have had no prior exposure to cytotoxic drugs (1). In addition, there is now evidence that adjuvant chemotherapy, given after mastectomy in patients who have involved axillary nodes, reduces the incidence of relapse in certain subgroups (2). Unfortunately, many regimens require frequent clinic visits for periods up to 1 year, although there are data supporting shorter courses of adjuvant chemotherapy (3–5). Toxic effects of therapy such as nausea, vomiting, and alopecia may be bearable for short periods but often become less tolerable with time, so the concept of a short intensive course of adjuvant chemotherapy, starting early in the postoperative period and being completed within 6 weeks, is attractive to both physician and patient. We report the results of a pilot study designed to test the efficiency and tolerance of such a combination in patients with metastatic disease.

TABLE 1.—Patient characteristics

No. of patients	
Premenopausal	10
Early postmenopausal (UICC)	2
Late postmenopausal	9
Median (range)—	
Age in years: 53 (31–64)	
Karnofsky performance status: 70% (50%–90%)	
Disease-free interval in mos: 33 (3–132)[*]	
Interval from 1st recurrence to chemotherapy in mos: 2 (0–27)[*]	
No. of patients with prior—	
Surgery only (mastectomy or biopsy)	11
Radiotherapy	5
Hormone therapy	
Tamoxifen	8[†]
Progestogen	2[‡]
Chemotherapy	0
No. of disease sites	
Primary tumor	6
Lung and pleura	14
Bone	13
Liver	5
Soft tissue	6
Nodes	11
Bone marrow	4
Brain	2

[*] Excluding 6 patients with unresected primary.

[†] 2 responses lasting 14 and 15 mos.

[‡] No response.

Patient characteristics are shown in table 1. The dominant sites of disease were primary tumor (six patients), lung (seven), liver (three), soft tissue and nodes (four), and bone (one). Disease was present at two sites in ten patients and at three or more sites in eight patients.

Chemotherapy (VAP-Cyclo) consisted of 50 mg/m^2 of doxorubicin iv and 500 mg/m^2 of cyclophosphamide iv in alternate weeks × 3; 1.4 mg/m^2 of vincristine weekly × 6; and 25 mg/day of prednisolone orally continuously for 6 weeks. Patients were reassessed at 8 weeks, and further treatment depended on the response to the induction course. The majority of patients received further chemotherapy with 50 mg/m^2 of doxorubicin iv and 500 mg/m^2 of cyclophosphamide iv every 3 weeks (A/C).

Twenty-one of the 25 patients entered in the study were considered to be evaluable. Of the four inevaluable patients, one had no clear evidence of disease progression 1 month after oophorectomy, and the remaining three received inadequate chemotherapy. Reassessment 2 weeks after the completion of VAP-Cyclo showed three patients to be in complete remission and six to be in partial remission by International Union Against Cancer (UICC) criteria: the overall response rate was 42.8%. One of the patients who had complete remission had cerebral metastases controlled by irradiation and dexamethasone in addition to a pulmonary metastasis which disappeared. Disease stabilization was observed in eight patients, and three had disease progression. There was one early death (26 days) due to toxicity.

The 17 patients showing remission or disease stabilization received further chemotherapy with A/C; the number of courses ranged from one to ten. During this therapy, three more patients achieved complete remission; two of the three had shown minor response, and the other had had a partial remission at the end of VAP-Cyclo. The durations of response for patients with complete remission are 8, 10, 13, 14, 13+, and 14+ months. Five patients remained in partial remission for 7, 7, 8, 10, and 12 months; four had stable disease for 2, 3, 5, and 7 months; and two had disease progression. The overall response rate to VAP-Cyclo and A/C was 52.4%. Two complete remissions were in lung, one was in lung and liver, one was in soft tissue, and the remaining two were regressions of large primary tumors. The dominant sites of disease in the five patients with partial remission were liver, primary tumor, soft tissue, bone, and lung (one patient each).

Toxicity (21 Evaluable Patients)

Dose modification.—Vincristine was omitted once in four patients and four times in one patient, usually because of mild weakness or severe constipation. Myelosuppression necessitated a delay of 1 week on one occasion in four patients and a delay of 1 week on two occasions in two patients.

Hematologic.—The mean wbc count nadir was 2.6 × 10^9/L (range, 0.6–6.2), with only three patients having a nadir $< 1.0 \times 10^9$/L. The mean platelet count nadir was 195 × 10^9/L (range, 25–216), with two patients having a nadir $< 50 \times 10^9$/L. Four patients with myelosuppression were known to have bone marrow infiltration. There were two significant infections requiring iv antibiotic treatment. One patient who had extensive liver, lung, and pulmonary metastases died after 26 days from septicemia and bleeding secondary to myelosuppression (wbc count nadir, 0.6 × 10^9/L; platelet count nadir, 50 × 10^9/L).

Nonhematologic.—Nausea and vomiting of varying severity occurred in all patients, but treatment modification was not required. Oral candidiasis occurred in seven patients and alopecia was universal. Significant peripheral neuropathy occurred in only one patient, although paresthesias in the extremities were common.

This study has shown that an intensive 6-week induction course of chemotherapy with VAP-Cyclo is active against metastatic breast cancer. The majority of patients in this study had a substantial tumor burden that would be unlikely to resolve completely after a 6-week induction course of chemotherapy. However, in the adjuvant situation, complete destruction of the systemic micrometastases of responsive tumors might be possible in a 6-week period, although this remains to be tested.

Combination VAP-Cyclo was generally well-tolerated and toxic effects were usually rapidly reversible. In fit patients with good hepatic and renal functions and normal bone marrow, this particular combination would probably be well-tolerated and would be acceptable as an adjuvant treatment if patients were carefully monitored during the 8-week, high-risk period.

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Vivien H. C. Bramwell
A. Howell
H. Scarffe
D. Crowther

Cancer Research Campaign
Department of Medical Oncology
Manchester University
Christie Hospital and Holt Radium Institute
Wilmslow Rd
Manchester M20 9BX
England

R. A. Sellwood

Department of Surgery
Withington Hospital
Nell Lane
Manchester M20 9BX
England

July 30, 1982

Subcutaneous Reaction Due to Intra-arterial Infusion of Doxorubicin

Intra-arterial infusion of doxorubicin is often used in the chemotherapeutic protocol for the treatment of some tumors (1). The toxic effects of doxorubicin include myelosuppression, cardiotoxicity, gastrointestinal toxicity, alopecia, and stomatitis (1–2). In addition, extravasation of doxorubicin may cause skin necrosis and ulcers (3). Skin discoloration occurring during intra-arterial infusion of doxorubicin and extending over the area of an osteosarcoma has not yet, to our knowledge, been described.

CASE REPORT

A $12\frac{1}{2}$-year-old girl was admitted to the hospital because of constant pain in her right knee. A biopsy established the diagnosis of an osteogenic sarcoma. Our treatment protocol included three courses of doxorubicin (60 mg/m^2) and cisplatin (100 mg/m^2) prior to surgery. While the patient was receiving the doxorubicin (100 mg in 100 ml of solution), a large (10 × 15 cm) brownish-purple area of discoloration was noted on the extensor surface of the right thigh. The infusion was discontinued. Extravasation was excluded by demonstrating angiographically that the tip of the catheter was in the femoral artery. The lesion was not associated with any neurologic impairment. A skin biopsy 1 month later showed intracellular and intercellular edema of the epidermis with pyknosis of the nuclei. Telangiectatic capillaries were present just below the flattened epidermis. This area of pigmentation has faded and decreased in size but has not completely disappeared even after 3 years.

DISCUSSION

To achieve high concentrations of cytotoxic drugs in limited anatomic areas occupied by tumor, regional chemotherapy is given by intra-arterial infusion. This method of treatment enables us to confine the drug to the involved area and therefore reduces the systemic side effects. In our patient we intended to perform limb salvage as the orthopedic procedure.

As no metastases were found, the goal of treatment was to decrease the tumor bulk before surgery. Doxorubicin was injected in the usual concentration into the femoral artery as verified by angiography. Haskell et al (4) described skin necrosis that developed 1 week after intra-arterial infusion of doxorubicin in a patient with esophagopharyngeal carcinoma. However, it occurred in an area that had previously received > 7000 rads of radiation therapy. Di Pietro et al (5) have described local reactions to intra-arterial infusion of doxorubicin, including thrombosis, occlusion, arteritis, necrosis of the skin, and neuritis, none of which were observed in our patient. Considering that neither malignant metastatic skin invasion nor skin necrosis was noted in this case, we presume that the high local concentration of doxorubicin per se was responsible for the local discoloration.

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A. Ballin
G. Kende
B. Morag
M. Ronen

Department of Pediatric Hematology
Chaim Sheba Medical Center
Tel-Hashomer
Israel

August 9, 1982

ANNOUNCEMENTS

Topics in Emergency Medicine

San Francisco, California, January 31–February 4, 1983

This course will be presented by Extended Programs in Medical Education, University of California School of Medicine, San Francisco and will take place at the Sheraton Hotel at Fisherman's Wharf. The program is designed to instruct emergency medical practitioners in depth through didactic presentations, case studies, student/teacher interactions, and demonstrations in the approach to problems in the emergency department. Upon completion of this program, registrants should be more competent in handling injuries of the hand and face, in diagnosing and treating acute cardiac disease, in interpreting radiographs, and in managing problems in other areas including pediatrics, ophthalmology, neurology, obstetrics/gynecology, and endocrinology. Pre and post course assessments will be available, as well as a syllabus with references.

This course meets the criteria for 35 hours of credit in the following: Category 1 of the AMA Physician's Recognition Award, the CMA Certification Program, the American Academy of Family Physicians, and Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, Rm 569-U, Third and Parnassus Ave, San Francisco, CA 94143 (telephone 415-666-4251).

Frontiers in Hematology/Oncology II: Complications of Neoplastic Disease in Therapy

Warren, Vermont, February 7–11, 1983

This meeting will take place at the Sugarbush Inn, Sugarbush Valley Ski Resort and will be presented in nine 2-hour sessions which will cover major topics of clinical interest to academic and practical hematologists, oncologists, and internists with special interest in hematology/oncology. Emphasis will be placed on review of the latest developments regarding the complications of treating patients with neoplastic diseases, pain control, infections in the immunocompromised host, newer antibiotics, and control of chemotherapy-induced toxicities. Credit for 20 hours of CME will be given. Registration fee is $275.

For more information, contact: Ms. Linda Bonacquisti, Albany Medical College, Division of Oncology, Albany, NY 12208 (telephone 518-445-5361).

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Seventh Annual Cell Kinetics Society Meeting

Baltimore, Maryland, March 16–19, 1983

The seventh annual meeting of the Cell Kinetics Society will be held at Baltimore's Hilton Hotel. The Cell Kinetics Society has an international membership of approximately 300 experimentalists, clinicians, and mathematical modelers who share an interest in the cell proliferation patterns of normal and abnormal tissues.

For more information concerning this meeting or membership, contact: Bruce F. Kimler, PhD, Secretary, Cell Kinetics Society, Department of Radiation Therapy, University of Kansas Medical Center, Rainbow Blvd at 39th St, Kansas City, KS 66103.

Neuro-oncology Fellowships

University of California, San Francisco, July 1983

Two neuro-oncology fellowships will be available in the Department of Neurological Surgery/Neuro-oncology Service at the University of California in San Francisco beginning in July 1983. The fellowships emphasize clinical and research training in primary adult and childhood tumors of the central nervous system. The first year is clinical and the second year is clinical or laboratory research. Candidates should be graduates of approved neurology or neurosurgical training programs. The University of California is an equal opportunity/affirmative action employer. Women and minorities are encouraged to apply.

For further information, contact: Dr. Victor A. Levin, Brain Tumor Research Center, 783 HSW, University of California Medical School, San Francisco, CA 94143.

Cancer Immunology Fellowship

Paris, France

A 2-year fellowship in clinical and laboratory cancer immunology is available at the Laboratoire Universitaire d'Immunobiologie des Tumeurs Humaines, and Chaire de Cancérologie. The program involves intensive training in medical oncology and clinical and laboratory work on human tumors, draining lymph nodes, thymus, spleen, and peripheral blood. Previous experience with monoclonal antibodies and/or HPLC techniques is preferable. Ability to communicate in French is necessary. Funds available are $1000 per month.

Candidates should send a curriculum vitae and an introductory letter from their laboratory director to: Professeur Lucien Israël, Hôpital Avicenne, Bobigny, 93000 France.

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NIH Publication No. 83-22

66/12

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1982

cancer treatment reports

December 1982
Volume 66
Number 12
Pages 2005-2149

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

HE 20.3160:

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Begin the article with a short summary.

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Submit an original and 3 copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

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Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names or The Merck Index* for proper nomenclature.

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Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

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Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. A second table will be permitted only when requested by the editorial board. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen,* or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints

Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

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Random Prospective Study of Cyclophosphamide, Doxorubicin, and Methotrexate (CAM) Combination Chemotherapy Versus Single-Agent Sequential Chemotherapy in Non-Small Cell Lung Cancer[1]

Lawrence H. Einhorn,[2,3,*] Stephen D. Williams,[3] Eugene E. Stevens,[3]
William H. Bond, and Linda Chenoweth[3]

A group of 104 patients with unresectable non-small cell lung cancer were randomized to receive combination chemotherapy with cyclophosphamide, doxorubicin, and methotrexate (CAM) or single-agent sequential chemotherapy with the same three agents. CAM combination chemotherapy produced a 22% objective response rate, including two complete remissions, compared to a 9% response rate, including one complete remission, produced by single-agent therapy ($P = 0.16$). The median survival time was 32 weeks (range, 3–116) for CAM, compared to 25 weeks (range, 4–179+) for sequential single agents ($P = 0.24$). Overall survival was 31% (1-year), 16% ($1^1/_2$-year), and 5% (2-year), with no difference between the study arms. Although there was no statistically significant survival advantage for the CAM arm, both arms had survival superior to that in historical controls, presumably because of better patient selection. This study indicates that cyclophosphamide, doxorubicin, and methotrexate are, at best, marginally active as single agents, and new drugs with more efficacy are needed before combination chemotherapy can be expected to result in any meaningful prolongation of survival in non-small cell lung cancer. [Cancer Treat Rep 66:2005–2011, 1982]

Lung cancer is a major national health problem and is the most frequent cause of cancer death in the United States. Some 120,000 new cases are expected in 1982, and approximately 90% of these patients will die from their disease.

Although there have been advances during the past 10 years with chemotherapy in small cell lung cancer, progress in non-small cell lung cancer (NSCLC) has been less obvious. A variety of combination chemotherapy regimens have resulted in objective response rates of 25%–50%; however, there is no conclusive evidence that survival is prolonged with combination chemotherapy compared to single-agent chemotherapy or supportive care alone. In 1978, the most common components of these published combination regimens were cyclophosphamide, doxorubicin, and methotrexate (1–3). The purpose of this study was to determine in a stratified, random prospective trial whether there was any advantage in objective response rate or survival for combination chemotherapy with cyclophosphamide, doxorubicin, and methotrexate (CAM) versus single-agent sequential chemotherapy, ie, single-agent cyclophosphamide until disease progression, then doxorubicin, and finally methotrexate.

MATERIALS AND METHODS

From July 1978 to April 1981, 104 patients with histologically confirmed NSCLC were entered in a random prospective study; the schema and dosages for their chemotherapy are given in figure 1. Eligibility requirements were a Karnofsky performance status (KPS) ⩾ 50 and no prior chemotherapy with any of the study agents. Patients were stratified according to KPS, limited versus extensive disease, and prior radiotherapy and/or chemotherapy. Limited disease was defined as

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[1] Received May 3, 1982; revised Sept 7, 1982; accepted Sept 21, 1982.

[2] Dr. Einhorn is an American Cancer Society Professor of Clinical Oncology.

[3] Department of Medicine, Indiana University Medical Center, Indianapolis.

**Reprint requests to:* Lawrence H. Einhorn, MD, Department of Medicine, Indiana University Medical Center, 926 W Michigan St, University Hospital, Rm 582-N, Indianapolis, IN 46223.

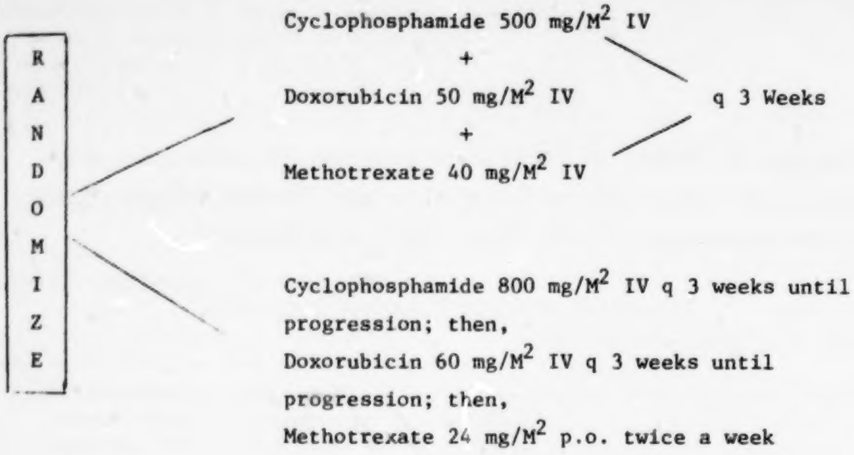
RANDOMIZE
Cyclophosphamide 500 mg/M² IV
+
Doxorubicin 50 mg/M² IV
+
Methotrexate 40 mg/M² IV
q 3 Weeks
Cyclophosphamide 800 mg/M² IV q 3 weeks until progression; then,
Doxorubicin 60 mg/M² IV q 3 weeks until progression; then,
Methotrexate 24 mg/M² p.o. twice a week

FIGURE 1.—Schema and dosages for random prospective study of CAM vs single-agent sequential chemotherapy with the same 3 agents.

disease confined to the hemithorax with or without ipsilateral cervical nodes.

CAM chemotherapy was continued until the total doxorubicin dose was 450 mg/m². Thereafter, chemotherapy was continued with cyclophosphamide plus methotrexate for a total of 1 year. Patients randomized to single-agent therapy received cyclophosphamide. After disease progression, the patient was switched to doxorubicin, and after further progression, to methotrexate. All therapy was done on an outpatient basis.

Patient characteristics are shown in table 1. The two groups were comparable, except for a slightly higher unfavorable KPS in the single-agent group and a higher percent of squamous cell lung cancer, but lower percent of large cell lung cancer, in the CAM group. This study was performed at our Veterans Administration Hospital (44% of the patient population) and county hospital (22%) as well as at our university hospital, and the percent of patients treated at each hospital was similar in both arms.

Pretreatment evaluation consisted of medical history; physical examination; cbc; SMA-12; posteroanterior and lateral chest X-ray; and radionuclide scanning of liver, bone, and brain. Tests of measurable disease parameters were repeated every 3 weeks. A complete response (CR) was defined as the disappearance of all clinical evidence of disease, and a partial response (PR) was defined as a 50% decrease in the sum of the products of the perpendicular diameters of measurable disease for at least 1 month or an estimated $\geqslant$ 75% decrease of poorly defined lesions in the absence of progressive disease elsewhere. Progressive disease was defined as the development of new lesions or a > 25% increase in measurable disease, and stable disease was defined as no remission or progression for a minimum of 3 months. Response duration was calculated from the time of remission to the time of disease progression, and survival was calculated from initiation of CAM or cyclophosphamide therapy to death.

Survival curves were drawn according to Kaplan and Meier (4) and were compared by Gehan's modified Wilcoxon test (5). Differences in proportions between the two groups were tested by the chi-square test (6).

TABLE 1.—Patient characteristics

Characteristic	Sequential single agents (53 patients)	CAM (51 patients)
Age in yrs		
Median	58	58
Range	31–81	42–73
% of patients with—		
KPS		
80–100	38	50
50–70	62	50
Histology		
Adenocarcinoma	36	26
Squamous cell carcinoma	42	69
Large cell carcinoma	22	5
Disease		
Limited	38	40
Extensive	62	60
Prior therapy		
Radiotherapy	13	12
Chemotherapy	8	10

Table 2.—Myelosuppression

	CAM	Single-agent therapy
Granulocyte count nadir (cells/mm^3)	1600	1700
< 1000 granulocytes	18%	16%
Platelet count nadir (cells/mm^3)	110,000	140,000

RESULTS

Fifty-one patients were randomized to CAM. Alopecia occurred routinely, and nausea and vomiting was common but not severe. Several patients had dose-limiting stomatitis requiring reduction of their methotrexate dosage. Myelosuppression was generally mild; however, four patients required hospitalization for granulocytopenic fever and presumed infection. There was one episode of fatal doxorubicin-induced congestive heart failure and one episode of hemorrhagic cystitis necessitating termination of cyclophosphamide. Toxic effects of sequential single-agent therapy were generally well-tolerated. Although there was equitoxic myelosuppression from therapy with CAM or single-agent cyclophosphamide, there was more significant alopecia and nausea and vomiting with the combination regimen. The myelosuppression data are shown in table 2.

The therapeutic results are shown in table 3, and the survival data are given in table 4. There was no statistically significant difference in response rate or survival in the two arms of this study. In the CAM group, the median survival time (MST) was 47 weeks for stable and responding patients and 10 weeks for patients with progressive disease. In the single-agent group, the MST was 56 weeks for stable and responding patients and 14 weeks for patients with progressive disease. The overall curve and the survival curves by response are shown in figures 2 and 3, respectively. There was a statistically significant survival advantage for responders and patients with stable disease versus those with progressive disease ($P < 0.0001$), and for responders versus patients with stable disease ($P = 0.002$). The relationship of response to histopathology is shown in table 5. There was no statistically significant advantage for either arm when histologies were compared.

The median duration of remission for the 11 responders receiving CAM was 30 weeks. There were only five objective responses to single-agent sequential therapy (four with cyclophosphamide and one with doxorubicin), with a median duration of 35 weeks. Thirty patients received second-line therapy with doxorubicin, with only a single partial remission of 15 weeks' duration. Only 11 patients received third-line therapy with methotrexate (no response), because most patients were not candidates for further therapy by the time their disease had progressed during the two prior chemotherapeutic trials. There are six patients still alive with CAM therapy (74+ to 104+ weeks from initiation of therapy), one of whom remains in continuous PR for 78+ weeks. There are six patients who remain alive with single-agent sequential therapy (58+ to 179+ weeks). One patient remains in continuous PR for 3+ years and has not received cyclophosphamide for 2 years, and one remains in CR for 2½ years. Thus, despite overall low response rates, there are individual patients in both arms who derived significant benefit from therapy.

The survival curves for limited and extensive disease are shown in figure 4. There was a statistically significant survival advantage for patients with limited disease ($P = 0.02$).

DISCUSSION

During the past 5 years, combination chemotherapy regimens have become increasingly popular in the treatment of inoperable NSCLC. Numerous programs utilizing cyclophosphamide and doxorubicin have yielded impressive objective response rates of 30%–50%, with median survival times of 6–9 months (1–3); updated results from the same institutions confirmed an apparent therapeutic superiority over older chemotherapy programs (7,8). To substantiate claims for an improvement in survival in NSCLC, reference is frequently made to the 3–5-month MSTs with supportive care alone in Veterans Administration Lung Study Group (VALSG) protocols of the 1960s (9) and with BACON (bleomycin, doxorubicin, lomustine, vincristine, and mechlorethamine) in the early 1970s (10). However, the VALSG protocols excluded nobody, and the BACON regimen was also used to treat all patients regardless of KPS; in fact, 11 of 38 patients (29%) with extensive disease

Table 3.—Therapeutic results

	Sequential single agents	CAM	P value
% CR + PR	9* (1 CR)	22 (2 CR)	0.16
Median duration of remission in wks (range)	35 (15–170+)	30 (10–78+)	0.36
% stable disease	36	39	0.96
% progression	55	39	0.23

*4 patients receiving cyclophosphamide, 1 receiving doxorubicin (15 wks).

TABLE 4.—Survival results

	Sequential single agents	CAM	*P* value
Median survival in wks (range)	25 (4–179+)	32 (3–116)	0.24
% 1-yr survival	29	33	0.28
% 18-mo survival	12	20	0.42
% 2-yr survival	6	4	0.97

had a KPS of 10–40, and only one patient had a KPS of 80–100. The KPS is probably the major prognostic variable affecting survival in NSCLC. All of the recent combination chemotherapy regimens exclude patients with KPS < 50. In fact, one recently published paper excluded patients with KPS of 10–50, age > 65 years, prior chemotherapy, or creatinine clearance < 65 ml/minute (11). Interpretation of therapeutic advantage compared to historical controls must be viewed with caution, if not skepticism.

Use of the CAP-I combination regimen (cyclophosphamide, doxorubicin, and cisplatin) has been duplicated by the Southeastern Cancer Study Group (SECSG) (12). There were only seven objective responses in 91 patients (8%); however, the MST of 6 months was identical to that found in the original CAP-I study by Eagan et al (1). MACC (methotrexate, doxorubicin, cyclophosphamide, and lomustine) was utilized by the Eastern Cooperative Oncology Group (ECOG), and only five of 43 patients (12%) responded, with an MST of 15 weeks (13), in contrast to the 44% response rate and MST of 33 weeks in studies in NSCLC by Chahinian et al (3,8). CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) was also studied by ECOG (14), and they achieved only a 22% response rate and an MST of 19 weeks, compared to the original CAMP studies that achieved a 36% response rate and an MST of 36 weeks (2,7).

The results of the present study compared CAM to single-agent cyclophosphamide in 104 patients. Patients who had disease progression while receiving cyclophosphamide were then switched to doxorubicin and then to methotrexate after further progression. All patients had a minimal follow-up of 1 year. There was no statistically significant survival advantage for CAM, but results in both arms were statistically superior to

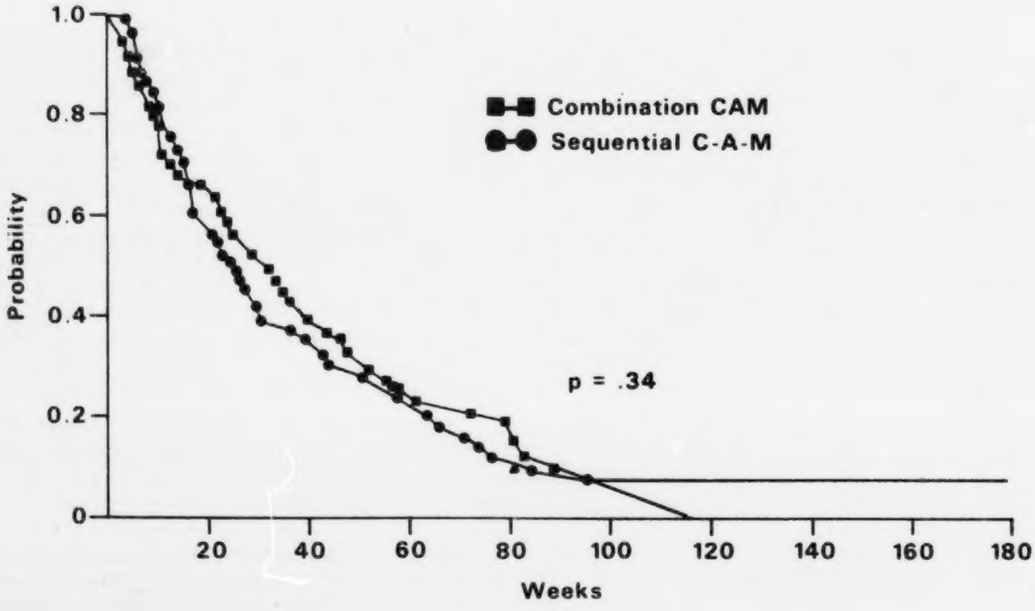

FIGURE 2.—Survival curves: CAM vs sequential single-agent cyclophosphamide, doxorubicin, and methotrexate.

TABLE 5.—Results by histology

Histology	Sequential single agents		CAM	
	Response*	MST (wks)	Response*	MST (wks)
Adenocarcinoma	1/19 (5)	26	2/13 (15)	34
Squamous cell carcinoma	2/22 (9)	19	8/35 (23)	28
Large cell carcinoma	2/12 (16)	40	1/3 (33)	46

*Values = No. of patients responding/No. in group (%).

those in VALSG historical controls, probably because of selection factors such as exclusion of patients with low KPS.

Although cyclophosphamide and doxorubicin have been incorporated in many combination chemotherapy regimens, they are probably, at best, marginally active agents. The Working Party for Lung Cancer compared COMB (cyclophosphamide, vincristine, semustine, and bleomycin) to single-agent cyclophosphamide in a random prospective study (15). Patient entry required histology of squamous cell carcinoma of the lung, no prior chemotherapy, serum creatinine < 1.6 mg/dl, and a Zubrod performance status < 4. Cyclophosphamide was given at a dose of 1100 mg/m^2 every 3 weeks to 27 eligible patients; there was only one objective response, and the MST was 14.4 weeks. Only one of 20 patients (5%) responded to COMB, and the MST on that arm was 10.9 weeks. The SECSG treated 91 patients with cyclophosphamide (500 mg/m^2) plus doxorubicin (50 mg/m^2) every 3 weeks (12). Eligibility requirements included creatinine clearance > 60 ml/minute and a KPS ⩾ 50. Only three patients (3%) responded; however, in this random prospective study, the MST of 6 months and the overall survival curve were identical to those for CAP-I (12).

Another random prospective study compared doxorubicin (40 mg/m^2) plus cyclophosphamide (700 mg/m^2) every 3 weeks to single-agent vindesine (D. Luedke, personal communication). A KPS > 50 and no prior chemotherapy were the eligibility criteria. The objective response rate was only 9% for doxorubicin plus cyclophosphamide versus 22% for vindesine alone in 59 evaluable patients.

Pannuti et al (16) compared cyclophosphamide (1000 mg/m^2) every 3 weeks to cyclophosphamide, methotrexate, 5-FU, and hydroxyurea in 58 patients with no

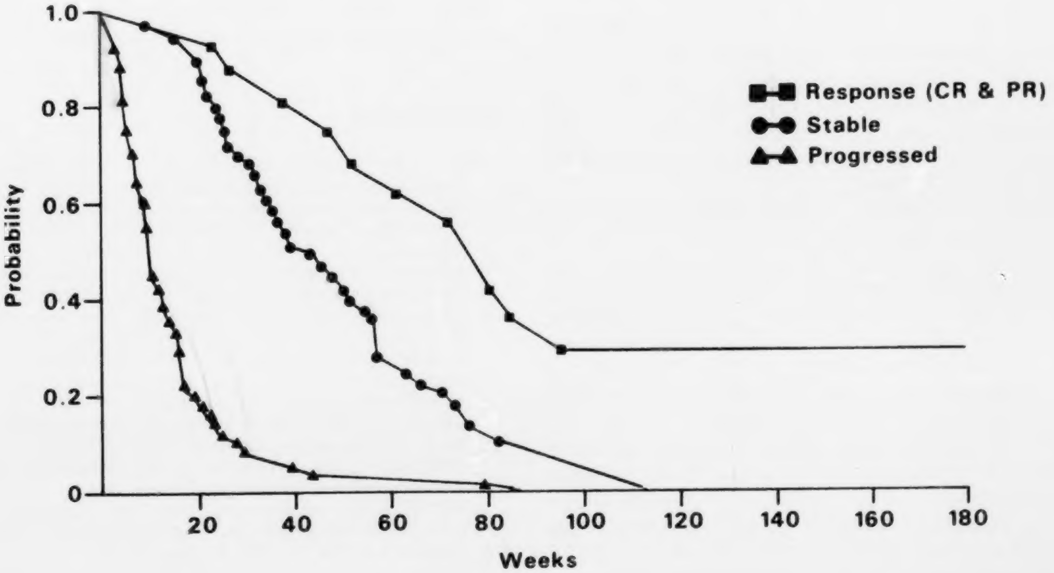

FIGURE 3.—Survival by response to therapy.

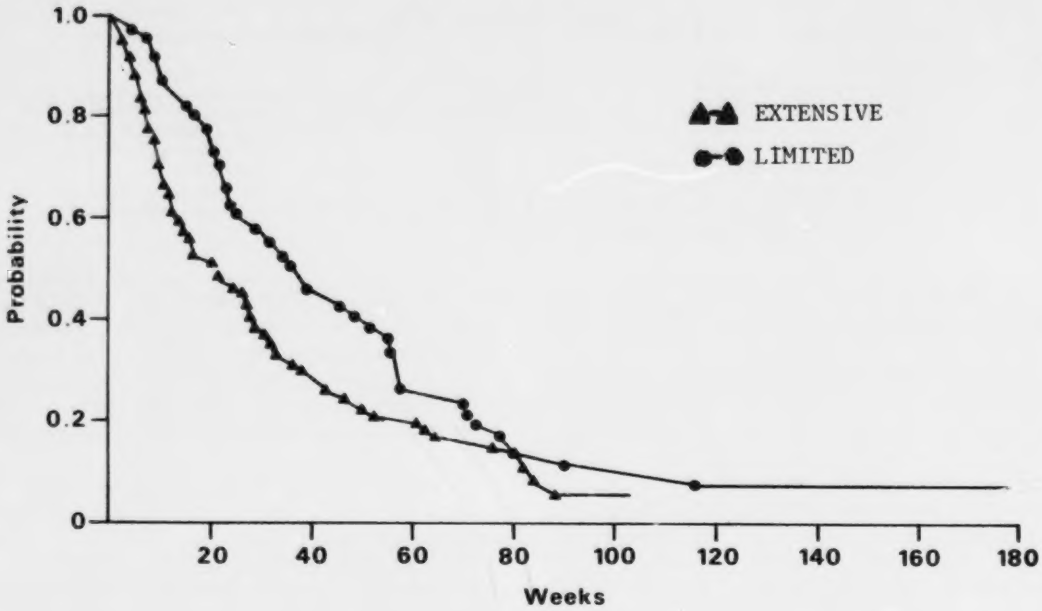

FIGURE 4.—Survival by extent of disease.

prior chemotherapy, KPS > 40, and age < 75 years. There was only one objective response in 29 patients (3%) treated with cyclophosphamide, with an MST of 24 weeks. The combination regimen had an identical 3% response rate with an MST of 26 weeks.

Davis et al (17) compared cyclophosphamide (600 mg/m^2) (41 patients) to cyclophosphamide plus lomustine and cyclophosphamide plus lomustine plus doxorubicin in 124 patients with extensive NSCLC, no prior chemotherapy, and KPS > 40. The response rates were only 5%, 8%, and 6%, respectively, with respective MSTs of 21, 17, and 19 weeks.

Finally, in our study, cyclophosphamide produced only four objective responses in 53 patients (8%). Even though doxorubicin was utilized as second-line therapy, it is difficult to be enthusiastic about its activity in NSCLC, since there was only one PR of 15 weeks' duration. Thus, these studies indicate that the objective response rate with cyclophosphamide is probably < 5% (eight of 179 responses), rather than the widely quoted 20%–30% response rate from the older literature.

Our major efforts in the past several years have been directed at utilizing phase II single agents as initial chemotherapy in inoperable NSCLC. Vindesine is thus far the only phase II agent we have tested that has significant single-agent activity. Six of 22 NSCLC patients (27%) with no prior chemotherapy achieved an objective response (18). The activity of vindesine is widely reproducible, with an overall objective response rate of 28% in 177 patients (18–23). However, more active agents will probably have to be found and utilized as components of a combination chemotherapy regimen before we can truly have an impact on survival in NSCLC. At Indiana University, we continue to evaluate phase II agents as initial chemotherapy for inoperable NSCLC.

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Cisplatin Therapy in Recurrent Childhood Brain Tumors[1]

Atiya B. Khan,[2,*] Bernard J. D'Souza,[2] Moody D. Wharam,[2] Lorraine A. A. Champion,[3] Lucius F. Sinks,[4] Shiao Y. Woo,[4] David C. McCullough,[4] and Brigid G. Leventhal[2,5]

Twenty-two patients with brain tumors were initially treated with surgery, radiation therapy, and/or adjuvant chemotherapy. Histologic diagnosis included 11 gliomas, seven ependymomas, one dysgerminoma, one teratocarcinoma, one pinealoma, and one small cell tumor. At recurrence, documented on computerized tomographic scan, cisplatin (60 mg/m^2/day iv × 2) was given every 3–4 weeks. Seventeen patients received two or more courses and were considered to be evaluable for response. Response parameters included change in the size of tumor on computerized tomographic scan and/or in clinical findings. Four patients had complete response, five had partial response, four had stable disease, and four had disease progression. Toxic effects were manageable. The number of patients with grade 3–4 toxic reactions, by category, were: thrombocytic, ten; leukocytic, four; renal, five; metabolic, four; gastrointestinal, none; and neurologic, seven. Two of the 14 patients tested had grade 3–4 ototoxicity. Acute fluid retention with decreased serum electrolytes and serious but reversible changes in the mental status, which were experienced in earlier patients, decreased in severity with increasing experience of the investigator. In summary, cisplatin appears to be active in a spectrum of brain tumors and should be studied further for therapeutic efficacy. [Cancer Treat Rep 66:2013–2020, 1982]

The current treatment for recurrent brain tumors poses a challenging therapeutic dilemma. Various chemotherapeutic regimens have produced varying degrees of response and often are only palliative. Cisplatin is a chelated heavy metal with proven antitumor activity in a wide spectrum of animal and human tumors (1–5). This report reviews the effectiveness and toxicity of cisplatin in recurrent brain tumors.

METHODS

The study group included 22 patients (11 from Johns Hopkins Hospital, ten from Georgetown University Hospital, and one from D. C. Children's Hospital). The histologic diagnoses were 11 gliomas (all grade 3 and 4), seven ependymomas, one dysgerminoma, one teratocarcinoma, one pinealoma, and one temporal lobe small cell tumor. Ages ranged from 4 to 22 years.

Patients were initially treated with surgery and radiotherapy (except for one patient), with or without adjuvant chemotherapy. Tumor recurrence was suggested by clinical deterioration and confirmed on computerized tomographic (CT) scan by either a new lesion (seven patients) or an increase in size of the pre-existing lesion (15 patients). Tumors recurred at the original site in all but six patients. Eight patients received additional radiotherapy and/or chemotherapy at the time of first recurrence but had either failed to respond or had tumor recurrence again before cisplatin was given (table 1).

Prior to initiation of therapy, all patients had a general physical and neurologic examination. Pretreatment laboratory studies included a cbc with differential and platelet counts, serum electrolytes, BUN, serum creatinine, 24-hour creatinine clearance, liver function tests, and an ECG. These studies, with the exception of the

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[1] Received Sept 2, 1981; revised Sept 9, 1982; accepted Sept 27, 1982.

[2] Departments of Pediatric Oncology, Neurology, and Radiation Therapy, The Johns Hopkins University, Baltimore, MD.

[3] Oakland Children's Hospital and Medical Center, CA.

[4] Department of Pediatric Oncology, Georgetown University Hospital, Washington, DC.

[5] We thank Ms. Carolyn Jones for her assistance in the typing and preparation of this manuscript.

Reprint requests to: Atiya B. Khan, MD, Department of Pediatric Oncology, The Johns Hopkins Oncology Center, Rm 3-121, 600 N Wolfe St, Baltimore, MD 21205.

TABLE 1.—Treatment and response among 22 patients with recurrent brain tumors*

Patient No.	Histologic diagnosis	Initial therapy			Disease recurrence		Therapy for recurrence prior to cisplatin	Time between last therapy and start of cisplatin	No. of cisplatin courses	Response/ duration (mos)†
		Surgery	RT	Chemotherapy	Time	Site				
1	Glioblastoma, right parietal temporal lobe	Partial resection	5500 R	BCNU	5 mos	Local	None	1 mo	3	PR/6
2	Glioblastoma multiforme, right parieto occipital region	Partial resection	5000 R	BCNU	6 mos	Local	PCNU × 3	4 mos	4	PR/5
3	Pontine-cerebellar astrocytoma	Biopsy	5000 R	None	$7^{1}/_{2}$ yrs	Cerebellum	Drainage of cyst and biopsy of underlying nodule	19 mos	8	SD/41
4	Thalamic glioma, grade 3	Partial resection	None	BCNU 14 mos	3 yrs	Local	VP shunt + 6000 R	7 mos	8	SD/41
5	Glioblastoma multiforme, thalamic region	Gross total resection	6000 R	VCR Pred CCNU 1 yr	$2^{1}/_{2}$ yrs	Local	None	35 mos	8	SD/24
6	Optic tract astrocytoma	Partial resection and shunt	5220 R	—	3 yrs		None	35 mos	1	NE/—
7	Mixed glioma, parieto-occipital, grade 3	Partial resection	5500 R	None	6 yrs	Local	Partial resection	6 yrs	2	PD/—
8	Pontine glioma	None	5500 R	None	6 mos	Local	—	6 mos	1	NE/—
9	Brain stem glioma	Biopsy	4500 R	—	$1^{1}/_{2}$ yrs	Right frontal area	2500 R	8 mos	1	NE/—
10	Glioblastoma multiforme, right temporoparietal	Biopsy	5500 R	—	2 mos	Local	Partial resection	5 mos	2	PD/—
11	Glioblastoma multiforme, left cerebral hemisphere	Partial resection	5000 R	—	?	Local	—	1 mo	1	NE/—
12	Ependymoma, right occipital	Partial resection	6000 R	—	2 yrs	Gross total resection	CCNU	10 mos	3	PD/—
					4 yrs	Partial resection	VCR			
					$4^{1}/_{2}$ yrs	Gross total resection	Procarb			
					$5^{1}/_{4}$ yrs	VP shunt				
13	Ependymoma, 4th ventricle	Partial resection + VP shunt	5000 R to brain, 3000 R to spine	MOPP 6 mos	1 yr	Local	Iv MTX	5 mos	1	NE/—

14	Ependymoma, 4th ventricle	Partial resection + VA shunt	5500 R	MOPP 6 mos	1 yr	Local	None	1 mo	7	SD/11
15	Ependymoma, 4th ventricle	Gross total resection	5500 R	None	1 yr	Cerebellum, lumbar spinal cord	RT to spine	14 mos	9	CR/10
16	Ependymoblastoma, 4th ventricle	Partial resection	5500 R + spinal RT	VCR $1^1/_2$ yrs	3 yrs		—	13 mos	2	PD/—
17	Ependymoma, 4th ventricle	Gross total resection	5100 R + spinal RT	Iv MTX + BCNU	1 yr	Pineal	Subdurals drained bilaterally	1 mo	8	CR/41
18	Ependymoma, 4th ventricle	Gross total resection	6000 R	VCR CCNU 18 mos	2 yrs	Local	—	1 mo	3	PR/3
19	Suprasellar dysgerminoma	Partial resection	5000 R	—	2 yrs		Partial resection + 4140 R	11 mos	7	CR/10
20	Small cell tumor, left temporal lobe	Gross total resection	5000 R	VCR 9 mos	1 yr	Right parietotemporal mass + spinal cord (C7-T3)	MOPP 3 mos (VCR 1 yr)	2 mos	6	PR/6
21	Pineal teratocarcinoma	Partial resection	5000 R	None	1 mo	Local	—	1 mo	6	PR/5
22	Pinealoma	Gross total resection	4500 R	VCR CCNU	4 mos	Right frontal	—	1 mo	2	CR/3

*VP = ventriculoperitoneal; VA = ventriculoatrial; RT = radiotherapy; BCNU = carmustine; VCR = vincristine; Pred = prednisone; CCNU = lomustine; MOPP = mechlorethamine, vincristine, procarbazine, and prednisone; MTX = methotrexate; Procarb = procarbazine; SD = stable disease; NE = not evaluable; PD = progressive disease.

†Duration of response from beginning of cisplatin treatment.

ECG, were repeated prior to every course. CT scan and hearing tests were done every 2–3 months. Informed consent was obtained from all patients.

All patients were hospitalized for treatment, which usually required a 3–4-day admission, with hydration and baseline toxicity studies on Day 1 and administration of medication on 2 subsequent days. Thorazine was administered 12 hours prior to cisplatin therapy and every 8 hours to alleviate nausea and vomiting. Iv hydration with 3000 ml/m^2/day of 5% dextrose in 0.45 normal saline plus 20 mEq/L of KCl was started 6 hours prior to cisplatin therapy. Cisplatin was administered iv at a dose of 60 mg/m^2/day × 2 every 3–4 weeks. Each vial (10 mg) was reconstituted with 10 ml of sterile water to a concentration of 1 mg/ml and was infused over 4–6 hours. The drug was protected from light to prevent decomposition. Iv hydration was continued with drug administration and for 14 hours after the cisplatin infusion was complete. Patients were closely monitored for signs of fluid and electrolyte imbalance. In an attempt to diminish toxicity, cisplatin was initially administered with mannitol and/or Lasix, but these drugs were not routinely given in later patients. Serum sodium and urine output were carefully monitored. Iv fluid administration was adjusted to maintain a serum sodium level > 135 mEq/L.

Steroid and anticonvulsant dosages were not altered during therapy. Nephrotoxic antibiotics such as tobramycin and gentamicin were avoided. Therapy was discontinued with evidence of progressive disease after two courses or if severe renal side effects or ototoxicity developed. Between courses, patients did not experience drug-related limitation of activity.

Response Criteria

Two parameters were used to evaluate response: (*a*) decrease in the size and degree of enhancement produced by tumor on CT scan as compared to CT scans before therapy with cisplatin, and (*b*) improvement in documented neurologic deficits.

Complete response (CR) was defined as the disappearance of any evidence of tumor on CT scan, with resolution of all neurologic deficits that were present before cisplatin therapy. Partial response (PR) was defined as a > 50% reduction in tumor size on CT, with resolution of some neurologic deficits. Stable disease was defined as no improvement in CT scan or neurologic status, but no progressive worsening documented on either examination. Progressive disease was defined as an increase in size of enhancing lesions on CT scan and/or deterioration in neurologic signs and symptoms.

Toxicity Criteria

Neurologic, gastrointestinal, hematologic, and renal toxic effects were graded according to the criteria set forth by the Cancer and Leukemia Group B. Criteria for metabolic toxicity and ototoxicity grading were devised on our own (table 2).

RESULTS

Among the 22 patients, five received only one course of therapy because of rapid clinical deterioration and could not be adequately evaluated for response. Four patients had a CR, five had a PR, four had stable disease, and four had progressive disease. The duration of response is defined by duration of clinical and/or CT scan improvement and ranged from 3 to 41 months (mean, 14). In all patients who had a positive response, improvement in the clinical neurologic examination preceded improvement on CT scan. A CR was observed in four patients after two to eight courses (median, seven). A PR on CT scan was observed in five patients after two to five courses (median, two). Significant neurologic improvement was observed in eight patients after one to five courses (median, two), and return to normal neurologic status was observed in five patients after three to eight courses (median, five).

TABLE 2.—Grading of metabolic toxicity and ototoxicity

Grade	Metabolic (mEq/L)		Ototoxicity
	Serum K	Serum Na	
0: No abnormality	3.5	135	No toxicity
1: Mild	3–3.4	130–134	Tinnitus
2: Moderate	2.5–2.9	125–129	High frequency hearing loss
3: Severe	2.0–2.4	120–124	Low frequency hearing loss (helped with a hearing aid)
4: Maximal/life-threatening	2	120	Profound deafness

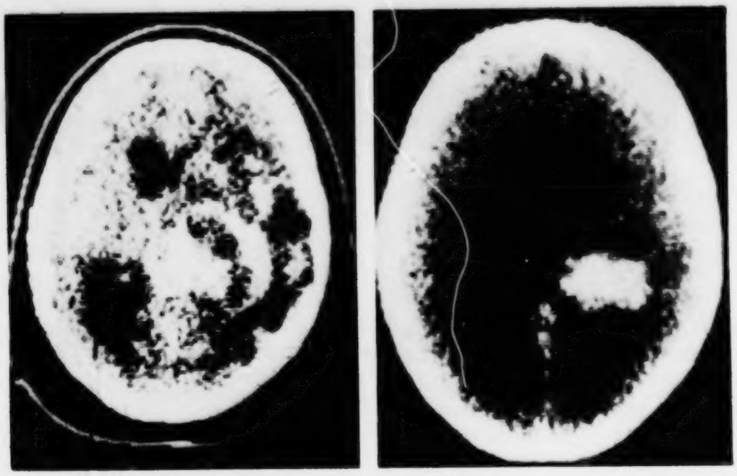

FIGURE 1.—PR of a glioblastoma to cisplatin in Patient 1. Scan on the left was obtained at recurrence, before cisplatin therapy. Scan on the right shows PR to therapy after 2 courses of cisplatin.

In the group of seven evaluable patients with gliomas, four tumors were located in the cerebral hemisphere, two in the thalamus, and one in the brain stem. None of these patients had a CR, but two (Nos. 1 and 2) had a PR (fig 1). Three patients (Nos. 3–5) had stabilization of disease and are alive 2–3$^1/_2$ years after treatment began. Two patients had progressive disease.

Four of the six evaluable patients with ependymomas had a favorable response. Two patients (Nos. 15 and 17) had a CR and are disease-free after nine and eight courses of treatment, at 1 and 3$^1/_2$ years after initiation of therapy (fig 2). Another patient (No. 18) showed definite improvement in the neurologic examination and on CT scan after two courses, but therapy had to be discontinued after three courses secondary to severe thrombocytopenia. This patient then had progressive disease and died. One patient (No. 14) has had stable disease for 11 months.

The remaining four patients with histologically small cell tumors (one pinealoma, one teratocarcinoma, one dysgerminoma, and one small cell tumor) all responded. Two patients (Nos. 19 and 22) had a CR (fig 3). One of

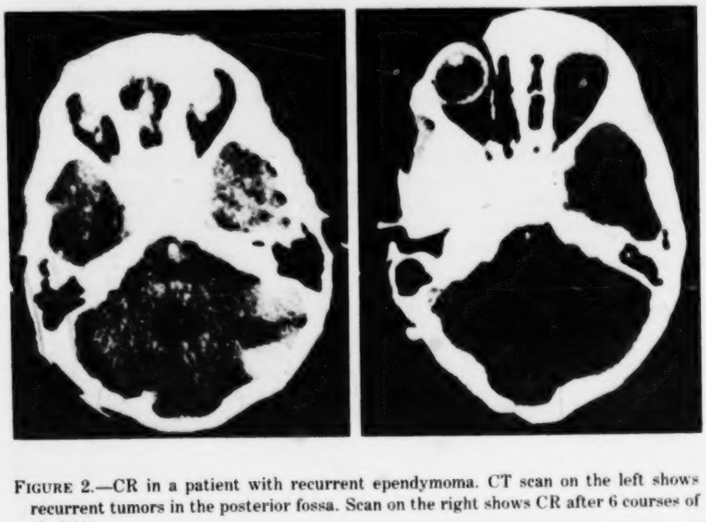

FIGURE 2.—CR in a patient with recurrent ependymoma. CT scan on the left shows recurrent tumors in the posterior fossa. Scan on the right shows CR after 6 courses of cisplatin.

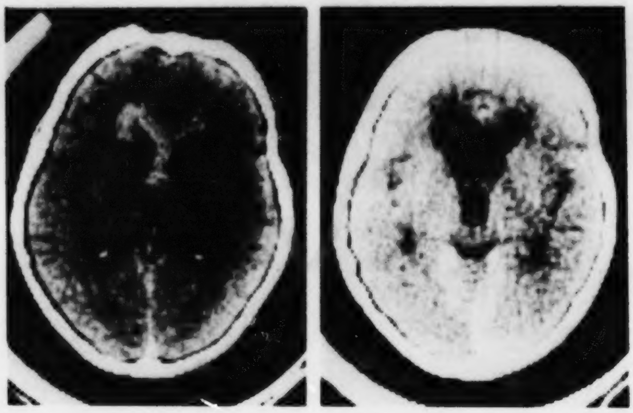

FIGURE 3.—CR of Patient 19. CT scan on the left shows paraventricular recurrence of suprasellar dysgerminoma. CT scan on the right is the appearance after 6 courses of cisplatin.

these patients (No. 19) later died of bilateral pneumonitis that was not biopsied, but lung cultures established at autopsy revealed *Staphylococcus aureus*. There was no evidence of tumor at postmortem examination. The other patient (No. 22), who showed a CR after two courses, developed renal toxic reactions necessitating discontinuation of treatment. This patient died following recurrence of her tumor. Two patients (Nos. 20 and 21) initially had a PR, but later had disease progression and were taken off therapy.

Five patients who died had autopsies. Two of these died while still responding. In both of these patients the autopsy findings confirmed those of the CT scan. One patient (No. 1) had a small 17-cm^3 tumor, which correlated with the 14-cm^3 volume calculated on the last CT scan (fig 1). The other patient (No. 19) had no tumor, but only necrosis around the lateral ventricles where tumor had previously enhanced. Three patients who were not evaluable for response were also autopsied. Two of these had progressive disease and one had hemorrhage in the tumor.

Toxicity

Table 3 documents the toxic effects seen in all 22 patients. Ten patients developed grade 3–4 platelet toxicity (< 75,000 cells/mm^3). One patient (No. 1) developed CNS bleeding 2 weeks after cisplatin administration. His platelet count was 11,000/mm^3 at that time. He did not have this toxic reaction with further treatment; perhaps initial sensitivity was due to a cumulative effect from his previous therapy with carmustine (BCNU). He improved with platelet transfusion and other supportive therapy. Four patients had

TABLE 3.—Toxic effects among 22 patients treated with cisplatin

	No. of patients by toxicity grade				
	0	1	2	3	4
Hematologic					
Platelets	8	1	3	3	7
Wbcs	10	3	5	2	2
Renal					
Creatinine	12	5	0	5	0
Metabolic	8	4	6	2	2
Gastrointestinal	0	10	12	0	0
CNS					
Hearing	2	0	10	2	0
Neurologic	15	0	0	5	2

severe granulocytopenia (< 1000 cells/mm^3). Two of these patients died: one of septicemia secondary to candida and klebsiella and one of salmonella sepsis. Patients who experienced moderate to severe hematologic toxicity early in the course of treatment had previously received other antineoplastic agents, and this could have contributed to the early onset of hematosuppression. Seven patients had also received spinal radiation, but there was no correlation between spinal radiation and early hematologic toxicity. Two deaths (Patients 1 and 19) secondary to pneumonia occurred at times when wbc counts were normal.

No patients developed grade 4 renal toxicity, but five had grade 3 (creatinine > 2 mg/100 ml or creatinine clearance < 75 mg/ml/min), necessitating discontinuation of treatment. None of the patients had grade 2 toxic reactions and five had grade 1. Twelve patients had normal renal function.

All patients experienced nausea and vomiting. None of the patients experienced grade 3–4 gastrointestinal toxicity, but 12 had grade 2.

Only 14 patients had serial hearing tests. Grade 2 ototoxicity was found in ten of these patients. Two patients had grade 3 hearing deficits requiring hearing aids. No ototoxicity was observed in two patients.

Of seven cases of grade 3–4 neurotoxicity, five were attributable to chemotherapy. Three patients (Nos. 6, 12, and 19) had seizures related to low serum sodium, which resolved with normalization of the serum sodium level. One patient (No. 1) had intracranial bleeding secondary to thrombocytopenia produced by cisplatin. This patient recovered with supportive treatment and did not have similar toxic reactions with repeated treatment. One patient (No. 9) developed respiratory difficulty and required mechanical support 24 hours after receiving cisplatin. This complication may have been due to the rebound effect of mannitol, which this patient had received with his cisplatin. He responded dramatically to steroids. Two other patients (Nos. 20 and 21) had a deterioration in their neurologic status during therapy, which was presumably related to progressive disease. The remaining 15 patients had no neurologic problems.

DISCUSSION

The results of our study indicate that cisplatin is active in a spectrum of brain tumors in children and is at least as promising as any other chemotherapeutic agents employed today. Past treatment of recurrent gliomas has resulted in a 40%–50% response rate with the following single agents: BCNU (6), lomustine (CCNU) (7), procarbazine (8), vincristine (9), teniposide (10), and high-dose methotrexate (11). Of our seven evaluable glioma patients, two showed PR, and three have had no progression of disease and are alive 2–3½ years after therapy (table 4). With ependymomas we have experienced even better results: two CR, one PR, and one stabilization of disease in six evaluable patients. Other drugs that have been used singly and in combination to treat ependymoma include vincristine, methotrexate (both intrathecal and systemic), BCNU, CCNU, procarbazine, and teniposide. Results in all these studies were similar, with response rates of 30%–50% (8–10,12–17).

All four evaluable patients from the miscellaneous tumor group had PR or CR. We are encouraged by the relatively high response rate to cisplatin in this group of tumors.

Gastrointestinal, renal, audiologic, hematologic, and neurologic toxic effects continue to be of major concern. Gastrointestinal toxicity, in particular nausea and vomiting, was the most distressing symptom to patients and necessitated the use of antiemetics with varying degrees of success. Robins et al (18) have demonstrated a safe and positive response with the combination of iv diazepam and prochlorperazine (Compazine) in 24 patients. This approach was not attempted in our study because of the high incidence of prochlorperazine reactions in children. Renal toxicity secondary to tubular damage is cumulative and seems to become evident after a total dose of 500 mg/m^2. In our patient population,

TABLE 4.—Summary of response to cisplatin

Tumor type	No. of evaluable patients	No. of patients with— CR	PR	Stable disease	Progressive disease	Duration of response (mos)
Glioma	7	0	2	3	2	5–41
Ependymoma	6	2	1	1	2	3–41
Pinealoma	1	1	0	0	0	3
Teratocarcinoma	1	0	1	0	0	5
Small cell tumor	1	0	1	0	0	6
Suprasellar dysgerminoma	1	1	0	0	0	10
Total	17	4	5	4	4	

ototoxicity secondary to cochlear damage was also cumulative and was first noted after 300 mg/m^2. In patients treated with cisplatin for other tumors, ototoxicity has not occurred this early. This early ototoxicity may be due to radiation therapy given to the brain in this group of patients. Two patients with severe hearing loss had significant improvement with use of a hearing aid. Recently, evidence for vestibular toxicity was reported in one patient (19). We had not performed vestibular function tests in our patients. Neurologic toxicity is probably related to fluid overload, hyponatremia, and hypomagnesemia and should be preventable. Clinical evidence for peripheral neuropathy was not observed in our study population. Long-term effects of this therapy are not known but are being assessed in the few patients who are still alive (> 1 year) after completion of therapy.

Although the number of patients treated in our study is small, these preliminary results suggest that cisplatin is an active agent in a spectrum of recurrent brain tumors. Stewart et al (20) measured cisplatin concentration in brain tissue, tumor tissue, and cerebrospinal fluid and reported that cisplatin selectively goes to tumor tissue, probably due to disruption of the blood-brain barrier. This selective uptake of platinum by tumor tissue is consistent with observed clinical effects. Additionally, cisplatin may prove to be more effective if used as an adjuvant to radiotherapy in view of the recent evidence that it may play a role as a radiosensitizer (21). The optimal duration of therapy and the long-term effects of cisplatin on normal CNS tissue have not been determined. Cumulative toxicity to the cochlea and the distal tubules of the kidney may well be the dose-limiting factor. An extended study using cisplatin in brain tumors is warranted.

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Comparative Trial of Two Teniposide-Based Combination Chemotherapy Regimens for the Treatment of Advanced Malignant Lymphomas[1,2]

Michael J. O'Connell,* James Anderson, John M. Merrill, John M. Bennett, John H. Glick, Costan W. Berard, Richard S. Neiman, and Harvey S. Brodovsky[2]

One hundred and twenty-three patients with advanced measurable malignant lymphomas resistant to conventional chemotherapy were entered in a prospectively randomized trial of two teniposide (VM-26)-based combination chemotherapy regimens: V-PLAT (VM-26, cisplatin, and prednisone) and V-HEX (VM-26, hexamethylmelamine, and prednisone). Ninety-seven eligible and evaluable patients received protocol therapy. Sixteen percent of the patients had Hodgkin's disease, and 84% had non-Hodgkin's lymphoma. All patients were ambulatory (Eastern Cooperative Oncology Group performance status 0, 1, or 2), 70% had stage IV disease, 59% had "B" symptoms, and all had failed either two or three previous chemotherapy regimens. Toxicity was mainly hematologic and significantly greater with V-PLAT. Objective tumor responses were seen in nine of 45 patients (20%) treated with V-PLAT (duration, 4–35+ weeks) and in four of 51 patients (8%) treated with V-HEX (duration, 10–65+ weeks). Among the 12 patients with histologically confirmed histiocytic lymphoma treated with V-PLAT, five (42%) experienced objective tumor responses, including two complete responses. Overall median survival was approximately 6 months, with no difference between treatment regimens. Limited antitumor activity of these combination regimens in patients with advanced malignant lymphomas has been demonstrated. However, the objective response rates were not higher than we have previously seen with either VM-26 (22%) or hexamethylmelamine (27%) given in maximum tolerable doses as single agents. [Cancer Treat Rep 66:2021–2025, 1982]

Although chemotherapy has the well-documented potential for cure of disseminated malignant lymphomas, many patients will develop drug resistance to conventional chemotherapeutic agents. Thus, there is a need for non-cross-resistant chemotherapy regimens for patients with refractory lymphoma who have been treated with alkylating agents, vinca alkaloids, anthracyclines, and other commercially available cytotoxic drugs.

The Eastern Cooperative Oncology Group (ECOG) elected to study two teniposide (VM-26)-based combination chemotherapy regimens in patients with refractory lymphoma to assess their antitumor activity in patients previously exposed to systemic chemotherapy.

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[1] Received May 19, 1982; revised Sept 13, 1982; accepted Sept 21, 1982.

[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Mayo Clinic, Rochester, MN (*M. J. O'Connell*, CA-13650); Sidney Farber Cancer Institute, Boston (*J. Anderson*, CA-23318); Northwestern University Medical Center, Chicago, (*J. M. Merrill*, CA-17145); University of Rochester Cancer Center, NY (*J. M. Bennett*, CA-11083); Hospital of the University of Pennsylvania, Philadelphia (*J. H. Glick*, CA-15488); St. Jude Children's Research Hospital, Memphis (*C. W. Berard*); Mallory Institute of Pathology, Boston, (*R. S. Neiman*); and Jefferson Medical College, Philadelphia (*H. S. Brodovsky*, CA-14215). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia (CA-18281); Baystate Medical Center, Springfield, MA (CA-20182); Brown University, Providence, RI (CA-15947); Case Western Reserve University, Cleveland (CA-14548); Chicago Medical School, IL (CA-14144); Hahnemann Medical College, Philadelphia (CA-13611); Johns Hopkins Oncology Center, Baltimore (CA-16116); Medical College of Ohio, Toledo; University of Minnesota, Minneapolis (CA-20365); New York University Medical Center, New York (CA-16395); Rush-Presbyterian-St. Luke's Medical Center, Chicago (CA-10948); University of Pretoria, South Africa (CA-21692); SUNY-Stony Brook (Veterans Administration Hospital), Northport, NY (CA-20161); Tufts University, Walpole, MA (CA-07190); Natalie Warren Bryant Cancer Center, Tulsa, OK; Medical College of Virginia, Richmond (CA-10572); Wisconsin Clinical Cancer Center, Madison (CA-21076); and Yale School of Medicine, New Haven, CT (CA-15485).

**Reprint requests to:* Michael J. O'Connell, MD, Department of Oncology, Mayo Clinic, Rochester, MN 55905.

The epipodophyllotoxin VM-26 was chosen based on previous ECOG experience (1) in patients with advanced lymphoma, which demonstrated objective tumor response in eight of 36 patients treated with VM-26 as a single agent, as well as on other reports in the literature of antitumor activity against malignant lymphomas (2–5).

Cisplatin was selected to be combined with VM-26 based on its reported single-agent activity in patients with advanced malignant lymphomas (6–8), its unique chemical structure which is quite dissimilar from other chemotherapeutic agents currently used for the treatment of malignant lymphomas, and its relative lack of myelosuppression. A pilot study of the VM-26, cisplatin, and prednisone regimen (V-PLAT) was carried out in nine patients at the Mayo Clinic to establish the appropriate dosage levels of VM-26 which could be combined with moderate-dose cisplatin (45 mg/m^2 given over 3 days) in previously treated lymphoma patients. Leukopenia (wbc count nadir $< 2000/\text{mm}^3$) and thrombocytopenia (platelet count nadir $< 100{,}000/\text{mm}^3$) were observed in four of the nine patients at a VM-26 dose of 25 mg/m^2 daily for 3 consecutive days in combination with cisplatin at a dose level of 15 mg/m^2 daily × 3. Prednisone was given at an empiric dose of 100 mg/m^2 for 5 consecutive days because of its known antitumor activity against malignant lymphomas and its palliative effects on the systemic symptoms which frequently occur in patients with advanced lymphomas.

Hexamethylmelamine was chosen for study based on previous ECOG experience (9) in which nine of 36 patients with advanced lymphomas obtained an objective remission when the drug was given as a single agent. The daily oral dose was decreased from 200 mg/m^2 × 21 days when given as a single agent to 150 mg/m^2 × 10 days when combined with VM-26 and prednisone in the VM-26, hexamethylmelamine, and prednisone regimen (V-HEX).

We used a randomized study design to minimize selection bias and to prospectively assess the relative antitumor efficacy of these two regimens in patients with advanced malignant lymphomas who had been previously treated with cytotoxic chemotherapy.

MATERIALS AND METHODS

All patients had a biopsy-proven diagnosis of malignant lymphoma of one of the following histologic types: Hodgkin's disease, nodular non-Hodgkin's lymphoma, or diffuse non-Hodgkin's lymphoma. Histologic diagnosis was confirmed by regional and/or national Panel For Lymphoma Clinical Trials review in 76% of the eligible patients. All patients had disseminated disease (stage III or IV according to the Ann Arbor classification) except one with progressive stage II disease following radiotherapy. Patients in a leukemic phase of their disease (as defined by the presence of circulating lymphoblasts in the blood) or > 75 years of age were not eligible for study. All patients had received at least one chemotherapy trial, with or without prior radiotherapy, and had failed or relapsed. Patients who had received any of the study agents, with the exception of prednisone, were excluded. All patients had at least one measurable indicator lesion to serve as an objective parameter of response to therapy. Radiographic findings were acceptable, provided clear-cut measurements could be made. Clearly defined defects measuring at least 5 cm in diameter on radionuclide, computed tomographic, or ultrasound examination of the liver were considered acceptable measurable parameters. Enlarged spleen and/or liver extending at least 5 cm below the costal margin on quiet respiration was also acceptable as a measurable parameter (biopsy proof of liver involvement by lymphoma was required if the liver was considered as the sole area of measurable disease). Patients had not received any cytotoxic chemotherapy or radiation therapy within 3 weeks of entry in the study (6 weeks for nitrosourea chemotherapy). Pretreatment laboratory test requirements included wbc count $> 4000/\text{mm}^3$, platelet count $> 100{,}000/\text{mm}^3$, total serum bilirubin < 2.0 mg/dl, and serum creatinine ≤ 1.5 mg/dl. Patients must have had an ECOG performance status of 0, 1, or 2. Patients with more advanced degrees of disease-related debility (performance status 3 or 4) were not eligible.

Patients were stratified according to histology (Hodgkin's disease, nodular non-Hodgkin's lymphoma, or diffuse non-Hodgkin's lymphoma) and performance status (ECOG performance status 0–1 or 2). They were then randomized to receive chemotherapy with either the V-PLAT or the V-HEX regimen.

VM-26 was given by slow iv infusion over a 30- to 40-minute period on Days 1, 2, and 3, and prednisone was given orally at a dose of 100 mg/m^2 daily on Days 1–5 in both regimens. In the V-PLAT regimen, cisplatin was given at a dose of 15 mg/m^2 on Days 1, 2, and 3. In the V-HEX regimen, hexamethylmelamine was given orally at a dose of 150 mg/m^2 daily on Days 1–10. Treatment was repeated every 28 days if the patient was stable or had experienced an objective tumor response. A provision was made for escalation of the VM-26 dose by 20% if no hematologic toxicity was observed from the preceding course of chemotherapy.

Patients were judged to have a complete response if there was complete regression of all palpable and X-ray-demonstrable disease. Partial response was indicated if there was at least a 50% reduction in the sum of the products of the perpendicular diameters of measurable lesions, or at least a 30% decrease in the linear measurements of liver or spleen. Furthermore, to qualify for either complete or partial response, there could be no decline in the performance status, no significant clinical deterioration, and the observed duration of response had to last for at least 4 weeks. Increase in liver or spleen size by at least 2 cm, symptomatic deterioration due to malignant lymphoma, or a decline in performance status of more than one level not explained by other medical conditions was considered disease progression.

Differences in toxicity by treatment were assessed using Fisher's exact test (10). Survival from the start of the study was estimated using the method of Kaplan and Meier (11). Comparisons of survival were made using the log-rank test (12).

RESULTS

Administrative Data

One hundred and twenty-three patients were entered in the study. Six patients (three randomized to each treatment group) were cancelled prior to beginning therapy. Fourteen patients (11 V-PLAT and three V-HEX patients) were found on non-blinded review of the records not to meet eligibility criteria for the following reasons: unacceptable pretreatment laboratory value (six patients), inadequate interval from previous oncologic therapy (four), lack of measurable indicator lesion (two), unacceptable pretreatment performance status (one), and age > 75 years (one). Five additional patients were excluded on the basis of pathology review (two V-PLAT and three V-HEX patients), indicating an ineligible histologic diagnosis. No records were received for one patient randomized to V-PLAT. Thus, there were 97 patients (46 treated with V-PLAT and 51 treated with V-HEX) available for analysis. Characteristics of the eligible patients who received protocol therapy are summarized in table 1.

Toxicity

Chemotherapy toxicity was primarily hematologic. Patients treated with the V-PLAT regimen experienced significantly greater hematologic toxic effects than did those treated with V-HEX. Among the 89 patients evaluable for hematologic toxicity, 31% treated with V-PLAT as compared to 6% treated with V-HEX had wbc count nadirs $< 2000/\text{mm}^3$ and/or platelet count nadirs $< 50{,}000/\text{mm}^3$ ($P < 0.01$). One patient treated with each regimen died of sepsis during a period of chemotherapy-induced leukopenia. A third patient (who was allergic to allopurinol) received treatment with V-HEX and died with acute renal failure and hyperuricemia, despite fluid administration and alkalinization, presumed to be secondary to tumor cell lysis. Six patients, all treated with the cisplatin-containing regimen (V-PLAT), experienced severe vomiting. Overall, treatment-related side effects were not considered to be excessive in this population of previously treated lymphoma patients.

TABLE 1.—Patient characteristics

	No. of patients	
	V-PLAT	V-HEX
Eligible for evaluation	46	51
Age in yrs		
< 40	13	10
40–60	18	24
> 60	15	17
Sex		
Male	24	31
Female	22	20
Histology*		
Hodgkin's disease	8	8
Nodular lymphoma	8	10
Diffuse lymphoma	17	25
Nodular and diffuse lymphoma	12	7
Unclassifiable lymphoma	1	1
Anatomic stage†		
III	11	17
IV	35	33
Symptomatic stage†		
A	15	24
B	31	26
Unknown	—	1
Performance status		
0	14	12
1	21	25
2	11	14
Prior chemotherapy		
2 regimens	21	31
3 regimens	24	20
Unknown	1	—
Prior radiotherapy		
1 course	22	28
2 courses	10	12
3 courses	13	11
Unknown	1	—

*Non-Hodgkin's lymphomas classified according to the Rappaport classification. Specimens with focal nodularity *and* diffuse pattern listed as "nodular and diffuse."

†Ann Arbor classification. 1 patient treated with V-HEX had progressive stage II disease in a previously irradiated site.

Tumor Response

A summary of the characteristics of patients experiencing objective tumor response is shown in table 2. Patients experiencing mixed response or transient antitumor effects lasting < 4 weeks were classified as nonresponders and were excluded from this table. One patient treated with V-PLAT was not evaluable for tumor response because of lack of serial tumor measurements. The overall objective response rate was 14% (13 responses among 96 patients). More patients responded to V-PLAT (20%, nine responses among 45 patients) than to V-HEX (8%, four responses among 51

TABLE 2.—Characteristics of responding patients

Patient No.	Regimen	Age (yrs)	Sex	Histology*	Stage	Performance status	Response†	
							Type	Duration (wks)
1	V-PLAT	45	F	DH	IV-B	2	CR	12
2	V-PLAT	53	M	DH	IV-A	1	CR	35
3	V-PLAT	65	F	Composite (NPDL; DH)	IV-B	2	CR	35+
4	V-PLAT	48	M	DH	IV-B	1	PR	21
5	V-PLAT	59	M	Nodular + diffuse histiocytic	IV-B	1	PR	6
6	V-PLAT	48	F	Nodular + diffuse mixed	III-B	1	PR	10
7	V-PLAT	61	F	NPDL	IV-B	0	PR	4+
8	V-PLAT	20	M	HD	IV-B	0	PR	8
9	V-PLAT	53	F	NM	III-A	1	PR	18+
10	V-HEX	54	M	DWDL	IV-A	0	CR	65+
11	V-HEX	67	M	DWDL	IV-B	1	PR	10
12	V-HEX	16	M	DM	III-B	1	PR	17
13	V-HEx	53	F	Nodular + diffuse lymphocytic	III-B	0	PR	27+

*DH = diffuse histiocytic lymphoma; NPDL = nodular poorly differentiated lymphocytic lymphoma; DM = diffuse mixed lymphoma; DWDL = diffuse well-differentiated lymphocytic lymphoma; HD = Hodgkin's disease; NM = nodular mixed lymphoma.

†PR = partial response; CR = complete response. Duration of response measured from first documentation of response to disease progression.

patients). This difference was of marginal statistical significance ($P = 0.08$). When response to treatment was evaluated according to histology, it was apparent that neither treatment was effective for patients with Hodgkin's disease (one response among 16 patients). Overall, objective responses were seen in two of 18 patients (11%) with nodular non-Hodgkin's lymphoma, in seven of 42 patients (17%) with diffuse non-Hodgkin's lymphoma, and in three of 19 patients (16%) with non-Hodgkin's lymphoma which demonstrated both nodular and diffuse growth pattern. The patient subgroup in which the most striking antitumor effect was noted included patients with histiocytic lymphoma treated with V-PLAT. Among the 12 patients with histiocytic lymphoma treated with V-PLAT whose histologic diagnosis was confirmed by pathology review (nine with diffuse histiocytic and three with nodular and diffuse histiocytic), five patients (42%) had an objective tumor response, including two complete responses.

Survival

There was no significant difference in survival, measured from the start of therapy, between patients receiving either of the chemotherapy regimens (fig 1). Median survival was approximately 6 months for both patient groups.

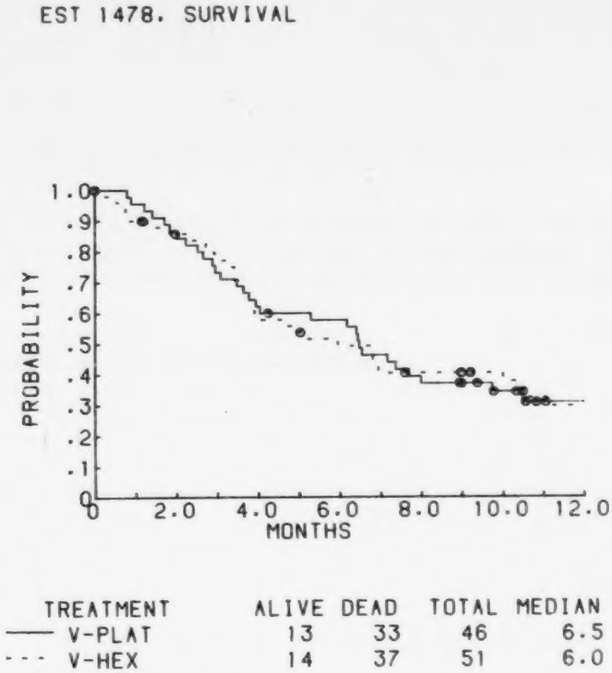
Analysis of the various patient and disease characteristics listed in table 1 indicated a statistically significant relationship between patient survival and anatomic stage ($P = 0.01$), symptomatic stage ($P = 0.002$), and pretreatment performance status ($P = 0.06$). Those patients with extranodal disease (stage IV), "B" symptoms, or impaired performance status had shorter survival. Patients with diffuse non-Hodgkin's lymphoma had somewhat shorter survival than did those with nodular non-Hodgkin's lymphoma or Hodgkin's disease, but this difference was not statistically significant ($P = 0.15$).

DISCUSSION

This investigation has documented limited antitumor activity for the two chemotherapy regimens studied in previously treated patients with advanced malignant lymphoma. Although the objective response rate observed with the V-PLAT regimen (20%) was higher than that seen with the V-HEX regimen (8%), this difference was of borderline statistical significance. Furthermore, the V-PLAT regimen was associated with more hematologic and gastrointestinal toxicity, and there was no difference in patient survival between the regimens.

These results should be considered in the context of previous ECOG experience in the treatment of advanced lymphoma patients with VM-26 or hexamethylmelamine as single agents, in which objective response rates of 22% (1) and 25% (9), respectively, were observed. Thus, neither of the VM-26-based combination regimens as given in this study produced an objective response rate superior to results of our previous experience with single-agent therapy. The failure to produce higher response rates with the V-PLAT or V-HEX regimen may in part be explained by the dosage reduction of VM-26 which was required in previously treated lymphoma patients with limited bone marrow reserves.

The high degree of antitumor activity of the V-PLAT regimen in heavily previously treated patients with histiocytic lymphoma in the present study (42% objective



TREATMENT	ALIVE	DEAD	TOTAL	MEDIAN
—— V-PLAT	13	33	46	6.5
- - - V-HEX	14	37	51	6.0

FIGURE 1.— Patient survival from initiation of protocol therapy, according to treatment regimen.

response rate among 12 patients) is of interest. In our previous study of VM-26 as a single agent in patients with refractory lymphoma (1), four of 12 patients (33%) with diffuse histiocytic iymphoma had objective responses, including two complete responses. Thus, VM-26 given alone or in combination with other agents may be particularly useful in the management of patients with histiocytic lymphoma.

The opportunity to more adequately evaluate the role of myelosuppressive agents such as VM-26 in the treatment of malignant lymphoma may require investigation in patients not previously treated with cytotoxic agents who would be expected to have more adequate bone marrow reserves. In addition, there would be considerable interest in further studies of nonmyelosuppressive agents in patients with refractory lymphomas to develop effective "second-line" regimens for previously treated patients.

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Phase I Trial of *Staphylococcus aureus* Cowan I Immunoperfusion[1,2]

Gerald Messerschmidt,[3,*] Charles Bowles,[4] Danny Dean,[3] Margaret Parker,[5] Robert Lester,[5] Regina Dowling,[3] Thomas Holohan,[3] Louise Osborne,[3] B. Frank Schaff,[3] Kathleen McCormack,[4] Robert Corbitt,[4] Terrance Phillips,[6] Eli Glatstein,[7] and Albert Deisseroth,[3,8]

Staphylococcus aureus **Cowan I has shown antitumor activity in in vitro and in animal tumor models. It is hypothesized that this antineoplastic effect results from the interaction of protein A on the cell surface of Cowan I strain *S. aureus* and immunosuppressive circulating immune complexes. Therefore, we treated five patients with ex vivo plasma immunoperfusion over killed and fixed *S. aureus* Cowan I. Toxic effects were marked in all patients and appeared to be related to the plasma volume infused and rate of infusion. Toxic reactions occurred in the cardiovascular, respiratory, and hematopoietic systems. No responses, even minimal or transient, were observed in this phase I trial. This toxicity may be reduced if the rate of plasma infusion decreases. [Cancer Treat Rep 66:2027–2031, 1982]**

Immune complexes have been implicated as one of the circulating plasma factors capable of suppressing cellular immunity in cancer patients (1). Protein A has the unusual property of binding free immunoglobulins and immune complexes by one of two Fc receptors within the molecule (2). Steele et al (3) hypothesized that the immune suppression could be reversed by removing immunosuppressive immunoglobulins by protein A found on the cell wall of *Staphylococcus aureus*. Studies demonstrated that the original immunosuppressive tumor-bearing rat sera no longer contained this capacity after exposure to *S. aureus* in vitro (3). Bansal et al (4), using a similar hypothesis, treated a patient with colon carcinoma with an ex vivo immunoperfusion system containing *S. aureus*. Changes were observed in the immune system and there was a decrease in tumor measurements. Subsequent animal studies with spontaneous adenocarcinoma treated with *S. aureus* immunoperfusion have demonstrated objective tumor responses in approximately 50% of the animals (5,6). Our group, therefore, applied this therapy to patients with cancer in a phase I trial.

MATERIALS AND METHODS

Patients with malignancy that had previously been treated with irradiation or chemotherapy and who had no standard curative therapy available were eligible for the trial. All gave informed consent. The first patient was treated in the Apheresis Unit of the National Cancer Institute (NCI). All subsequent patients were treated in the Critical Care Medicine Department (NIH). Access to the circulatory system was obtained by arterial or venous punctures and whole blood was withdrawn and separated into plasma and cellular elements by continuous flow centrifugation (AMINCO, Silver Spring, MD). The cellular elements were returned to the patient and the plasma was diverted to pass over a cartridge filter (Gelman Scientific, Ann Arbor, MI) which contained *S. aureus* (Pansorbin; Calbiochem, La Jolla, CA) at a dose of 1.0 g/kg of patient body weight. This amount of *S. aureus* was distributed in two to 14 cartridges. Plasma was pumped through the filters at 25 ml/minute. All patients treated in the Critical Care Medicine Department had continuous arterial pressure

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[1] Received June 24, 1982; revised Sept 13, 1982; accepted Sept 30, 1982.

[2] Presented in part at the American Society of Clinical Oncology meeting, Apr 26, 1982, St. Louis, MO.

[3] Experimental Hematology Section, Pediatric Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[4] Hazelton Laboratories, Vienna, VA.

[5] Critical Care Medicine, Clinical Center, National Institutes of Health, Bethesda, MD.

[6] Department of Medicine, George Washington University, Washington, DC.

[7] Radiation Oncology Branch, National Cancer Institute, Bethesda, MD.

[8] *Present address:* Hematology and Oncology, San Francisco Veterans Medical Center, CA.

[*] *Reprint requests to:* Gerald L. Messerschmidt, MD, Medical Oncology and Hematology Section, Department of Medicine, Wilford Hall USAF Medical Center, Lackland Air Force Base, TX 78236.

TABLE 1.—Patient characteristics

Patient No.	Diagnosis	Age (yrs)	Performance status
1	Synovial cell carcinoma	28	4
2	Colon adenocarcinoma	65	3
3	Esophageal carcinoma	33	3
4	Malignant melanoma	56	3
5	Esophageal carcinoma	63	4

monitoring and Swan-Ganz catheter monitoring performed. The patients were treated no more than once a week with no more than 2 weeks between perfusion treatments. We attempted to perfuse 100% of the calculated plasma volume. The plasma volume was calculated by the following equation: calculated plasma volume = (88 ml/kg) (100 − hematocrit) (body weight in kg). The plasma was anticoagulated with preservative-free heparin.

RESULTS

Five patients were treated. The patient characteristics prior to therapy are presented in table 1. Each patient had multiple courses of irradiation and chemotherapy prior to this trial. Patients 1 and 5 had severe pulmonary compromise as a result of tumor.

Eight immunoperfusion treatments were performed in these five patients. Table 2 outlines the procedure characteristics. We attempted to pass 100% of the calculated plasma volume through the cartridge filter containing the *S. aureus*. Because of severe toxicity and technical problems, only 24%–60% of the desired amount of plasma was perfused and returned to the patients. The technical problem that occurred with the cartridge filter system was filter plugging, necessitating manual pressure to pass the plasma through and return it to the patient. This occurred at some time with every procedure. Because of this technical problem and the toxicity, the plasma pump was turned on and off several times. Thus, the average plasma flow rate was less than that in the design, ranging from 3.4 to 15 ml/minute (table 2).

Toxicity

The toxicity of the procedure was excessive and resulted in termination of the protocol after the eighth perfusion procedure. Toxicity was manifested in the cardiovascular, respiratory, and hematologic systems. Figures 1–3 demonstrate the cardiovascular toxicity. Few changes were observed during the initial 100 ml of plasma infused. Following this amount, cardiac output and index increased in all patients but one (fig 1, A and B). Concomitant with this increase in cardiac function was an associated increase in mean arterial blood pressure (fig 2). The cardiac index and output remained elevated until the procedure was terminated and slowly returned to baseline by 8–12 hours. Mean arterial blood pressure began to fall as approximately 300 ml of perfused plasma was reinfused (fig 2), even as the cardiac output remained elevated. The fall in mean arterial pressure corresponded to a decrease in systemic vascular resistance (fig 3). Fluid administration was required in all eight procedures to maintain the mean blood pressure > 60 mm Hg. Vasoconstrictors were necessary in six of the eight procedures to maintain this level.

Because of the profound hypotension, the plasma pumps were turned off frequently. When the plasma reinfusion was interrupted, mean arterial blood pressure began to rise within 3–8 minutes. However, as the reinfusion was begun again, mean blood pressure fell again.

Respiratory rate increased by 25%–110% in these procedures. This was accompanied by a decrease in PaO_2 and $PaCO_2$ (fig 4, A and B). Two patients arrested and

TABLE 2.—Procedure characteristics with *S. aureus* immunoperfusion

Patient No.	Weight (kg)	Calculated plasma volume (L)	Plasma perfused and returned		Time (mins)	Average plasma flow rate (ml/min)
			Total ml	%		
1	60	3.5	1300	37	95	13.7
1	60	3.5	1003	29	75	13.4
2	54	3.3	1611	49	150	10.7
3	99	5.9	3517	60	235	15.0
3	99	5.9	2754	47	330	8.3
4	50	2.9	826	29	150	5.5
4	50	2.9	686	24	200	3.4
5	74	4.6	1190	26	90	13.2

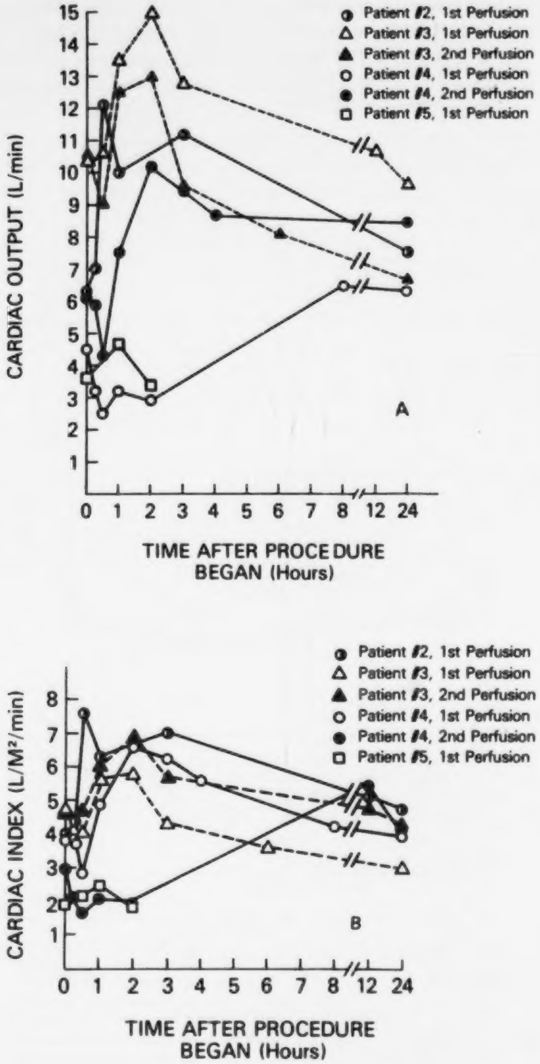

FIGURE 1.—A. Cardiac output. B. Cardiac index, in the 6 patient procedures performed in the Critical Care Department.

died as a result of respiratory problems. Both patients (Nos. 1 and 5) had severe pulmonary compromise prior to treatment. The first patient had approximately 75% of his chest volume obliterated by tumor and inaccessible fluid. Prior to his second procedure he had a PaO_2 of 54 mm Hg. At the end of the procedure, despite oxygen by mask, a PaO_2 of 29 mm Hg was measured, and he arrested and died. The fifth patient had esophageal carcinoma surrounding his trachea, resulting in severe extrathoracic airway obstruction. During this first procedure his respiratory rate increased to 44. As a result, he could not adequately ventilate, and he arrested and died. Autopsies performed on both patients failed to show any evidence of tumor response or to indicate the etiology of the cardiopulmonary response.

Hematopoietic toxicity was manifested in the total wbc and platelet counts. Total wbc counts decreased by 4 hours after the initiation of the procedure (fig 5A). By 8 hours, the total wbc count had returned to baseline or above. The decrease in total wbc count occurred as a resultant decrease in all cells, ie, granulocytes and lymphocytes. If an elevation over baseline occurred, this was most commonly associated with an increase in granulocytes and band forms. A normal differential count was observed by 48 hours. Platelet counts also decreased dramatically (fig 5B), again by 4 hours. However, unlike the total wbc count, it did not usually return to baseline for 48 hours following the initiation of the procedure. One patient required a 4-unit platelet transfusion for a platelet count of 17,000/mm^3.

No changes in hepatic or renal function could be attributed to this therapy. Identifiable toxic reactions were found only in the cardiovascular, pulmonary, and hematopoietic systems.

Tumor Response

In the eight procedures, four were associated with pain at the tumor site. However, no size decrease or change in these tumors could be documented.

DISCUSSION

Animal studies using ex vivo plasma perfusion over *S. aureus* have been very encouraging (5,6). In our animal studies, toxicity was minimal. As presented in this trial, man tolerates this therapy much differently than the dog, ie, with profound cardiorespiratory toxicity. This toxicity appeared to be related to the amount of the perfused plasma and the rate at which it was returned to the patients. That is, a reversal of the mean blood pressure fall began to occur within 3–8 minutes after the infusions were stopped, although complete reversal to baseline often did not occur for hours.

Although our animal trials were performed at a lower total dose of *S. aureus* (0.2 g/kg rather than 1 g/kg as in this trial), we do not believe this alteration plays a significant role in the observed toxicity. Our patients began to show cardiovascular and pulmonary responses with reinfusion of 100–300 ml of plasma exposed to *S. aureus*. Because of the technical problems of the filter chambers plugging, the total *S. aureus* (1 g/kg) was divided among several (two to 14) cartridges. This separation of the *S. aureus* now allows an examination of the role of the amount of active substance in the chambers and of this relationship to toxicity. We found that

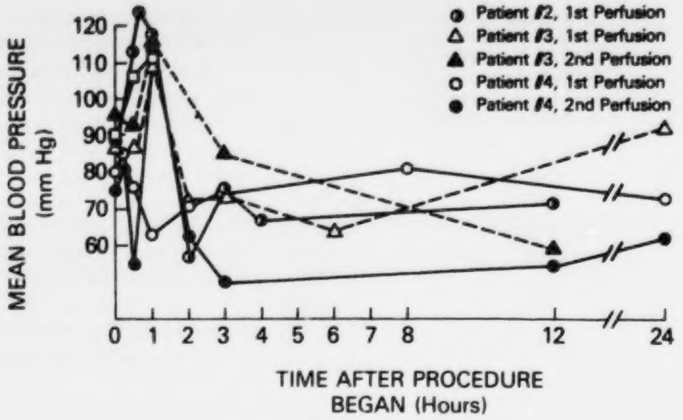

FIGURE 2.—Changes in the mean arterial blood pressure for each perfusion performed in the Critical Care Department, excluding the 5th patient who arrested during the 1st procedure.

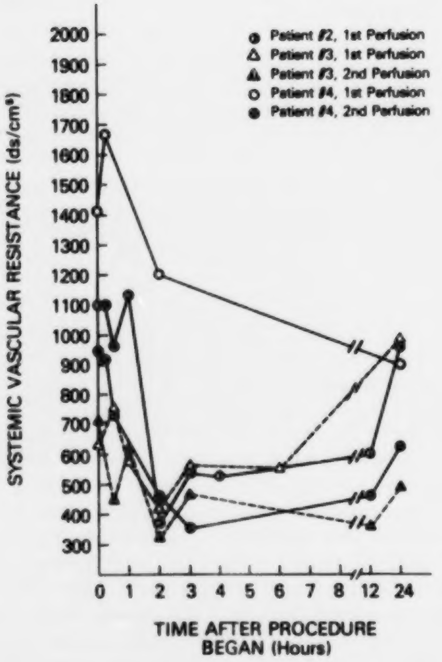

FIGURE 3.—Changes in systemic vascular resistance with time in 5 procedures performed in the Critical Care Department, excluding the 5th patient who arrested during the 1st procedure.

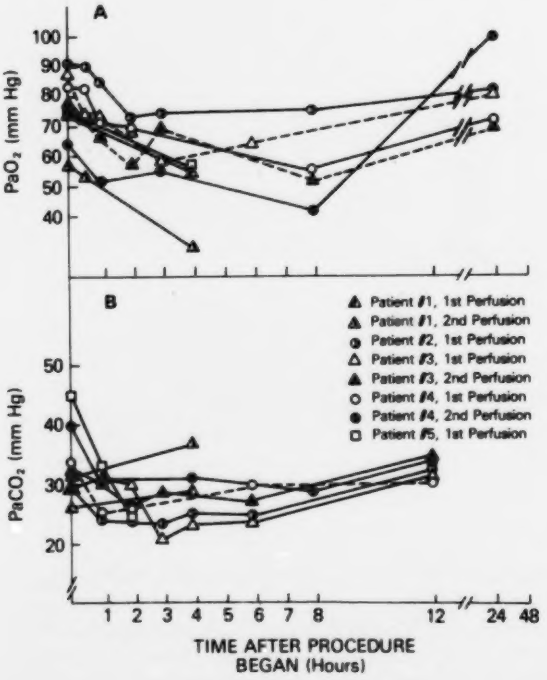

FIGURE 4.—A. Changes in PaO_2 in all patients treated with immunoperfusion with time. PaO_2 decreased in all procedures despite increases in respiratory rate. B. Changes in $PaCO_2$ in all patients treated with immunoperfusion. Decreases occurred in Patients 2–5 in conjunction with an increased respiratory rate.

the observable toxicity predictably began with 100–300 ml of infused plasma, which is the amount passed through the first cartridge only. The first cartridges contained between 0.5 g/kg (total, two cartridges) and 0.07 g/kg (total, 14 cartridges). Thus, the toxicity observed was present at levels well below those at which our dog trials were performed. It appears that toxicity correlates more closely with the amount of plasma exposed to the *S. aureus* and the rate reinfused, rather than the amount of *S. aureus* with which it comes into contact. These observations suggest that a product is generated or released in the ex vivo system that is related to the toxicity, and perhaps a slower rate of infusion would result in less toxicity. We are now examining the effluents in attempts to identify this product.

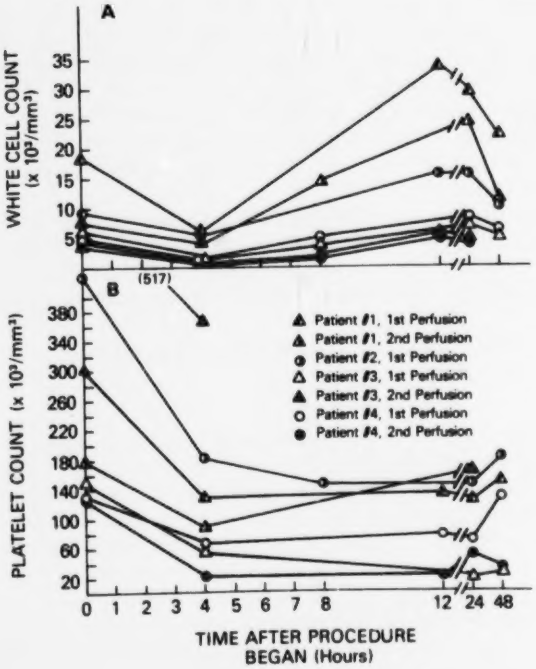

FIGURE 5.—A. Changes in total wbc count with time after initiation of *S. aureus* immunoperfusion. B. Changes in platelet count with time after initiation of *S. aureus*. 1 patient (No. 4) required a platelet transfusion to mantain a platelet count > 20,000/mm³.

The animal responses have been very encouraging; however, the use of *S. aureus* as a source of protein A in the treatment of human malignancy must be approached with caution. It may be possible to avoid this toxicity by slowing the infusion rate of the exposed plasma. Trials using the native *S. aureus* in the future should examine this approach and carefully monitor cardiac, respiratory, and hematopoietic toxicity.

Our animal data suggest that nearly 100% of the plasma needs to be perfused and reinfused for response (6); however, the toxicity observed in our patient procedures inhibited our ability to reach this amount of plasma. This, perhaps, is one explanation of why objective tumor responses were not observed in this phase I trial. If this toxicity can be avoided, then it may be possible to perfuse larger volumes of plasma and this may translate into tumor response as in our animal studies. Another alternative is to use purified protein A rather than the whole *S. aureus* in a similar perfusion system. Terman et al (7) have reported tumor shrinkage with less toxicity using such a system.

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Doxorubicin-DNA Complex: A Phase I Clinical Trial[1,2]

Stephen W. Hall,[3,4,*] Robert S. Benjamin,[3] Michael A. Burgess,[3] Gerald P. Bodey,[3] Mario A. Luna,[3] and Emil J Freireich[3,5]

Thirty-five patients with advanced malignancies were treated with doxorubicin-DNA complex in a phase I clinical trial. The patients ranged in age from 30 to 77 years (median, 51) and received doxorubicin-DNA in doses that ranged from 20 to 135 mg/m². Of the doses administered, 73% were 60–90 mg/m² (median, 60). Nonhematologic toxic effects included nausea and vomiting in 32% of the patients, fever and chills in 24%, mucositis in 18%, and decreases in ECG QRS voltage of > 30% in 18%. Fever of unknown origin was seen in 26% of the patients, with documented infection in 9%; 6% had drug-related deaths. All patients experienced alopecia. Hematologic toxic effects (median day, Day 14) included median granulocyte count nadirs of 0.8 × 10³/μl at a 60-mg/m² dose and 0.3 × 10³/μl at 75 mg/m². Thrombocytopenia was less severe, with median nadir counts of 131,000 cells/μl at a 60-mg/m² dose on median day, Day 13. Treatment courses could be repeated every 3 weeks. Clinical responses (complete plus partial) were seen in 12% of the patients, with an additional 48% showing stable disease. No patients entered in the study were known to be doxorubicin-resistant. Based on these data, we recommend a starting dose of 60 mg/m² in patients with adequate bone marrow reserve and 50 mg/m² in those with compromised bone marrows. Doxorubicin-DNA exhibits major clinical toxic effects on the gastrointestinal and hematopoietic systems, with dose-limiting granulocytopenia. [Cancer Treat Rep 6:2033–2037, 1982]

Doxorubicin is one of the most active antitumor agents in man (1). It has a broad spectrum of anticancer activity; however, its maximum cumulative dose is limited by cardiotoxicity. The occurrence of doxorubicin cardiomyopathy currently limits the clinical use of the drug to approximately 550 mg/m² total dose, which generally occurs after 20–45 weeks of therapy (2). Despite intensive investigation of the cardiotoxicity of doxorubicin, no effective means has yet been devised that allows a significant increase in drug dose or duration of therapy without undue risk of cardiac toxicity.

In 1972, Trouet et al (3) proposed the concept of lysosomotropic chemotherapy. Their hypothesis was that a drug could be bound to a digestible carrier, taken up selectively only into cells with the capacity for endocytosis; be concentrated within lysosomes; and following digestion, be released within the cell. This approach to drug therapy would avoid drug distribution throughout the body fluids and tissues from passive diffusion. Drugs with cardiac toxicity seemed appropriate to study in this manner, since it was felt that cardiac muscle cells have low pinocytotic activity. Antitumor drugs were also attractive because some cancer cells have increased pinocytotic activity (4). The anthracycline antibiotics fit these criteria very well and, in addition, bind avidly to DNA. Theoretically, the anthracycline-DNA complex would be excluded from cardiac muscle cells and taken up by neoplastic cells. Once inside the

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[1] Received Nov 2, 1981; revised Sept 17, 1982; accepted Sept 28, 1982.

[2] Supported in part by Public Health Service grant CA-05831 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and grants from Adria Laboratories, Wilmington, DE, and the Reno Cancer Center, NV.

[3] Departments of Developmental Therapeutics (*S. W. Hall, R. S. Benjamin, M. A. Burgess, G. P. Bodey,* and *E. J Freireich*) and Anatomical Pathology (*M. A. Luna*), the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[4] *Present address:* Department of Medicine, University of Nevada School of Medicine, Reno.

[5] We thank Dr. John Benvenuto, Ms. Judy Primeaux, and Ms. Charann Gamble for their assistance in the preparation of the drug.

[*] *Reprint requests to:* Stephen W. Hall, MD, Department of Medicine, Division of Oncology/Hematology, Veterans Administration Medical Center, 1000 Locust St, Reno, NV 89520.

cells, the drug would be concentrated in the lysosomes where endonucleases would degrade the DNA, releasing the anthracycline to do its cellular damage.

Early experimental animal studies with doxorubicin-DNA complex suggest increased therapeutic ratios and decreased acute cardiac toxicity (5). Preliminary human clinical studies with anthracycline-DNA complexes have suggested patterns of toxicity and efficacy similar to those observed with the free drugs, with one notable exception, a lack of cardiac toxicity (6). This study is a report of our clinical phase I trial of doxorubicin-DNA complex.

MATERIALS AND METHODS

Drug Preparation

Doxorubicin-DNA complex was prepared precisely according to the method of Trouet et al (7) using herring sperm DNA dissolved in normal saline at a concentration of 2.34 g/L. The DNA solution was autoclaved within 24 hours of administration and doxorubicin was added (1 mg/5 ml of DNA-saline solution) just prior to administration. The drug solution is stable with ~ 1.5% degraded by plasma deoxyribonucleases each hour (8).

Phase I Study in Patients With Solid Tumors

Thirty-five patients, 15 males and 20 females ranging in age from 30 to 77 years (median, 51), with disseminated solid tumors, were entered in this study. The patients' clinical diagnoses are outlined in table 1. Five patients were previously untreated. The rest were refractory to one or more prior chemotherapeutic regimens, and 13 (37%) had received radiation therapy as well. Eleven patients (31%) had received prior doxorubicin; however, four of these had received only one or two doses in the weeks immediately preceding the doxorubicin-DNA complex administration and were considered to be untreated with doxorubicin in the analysis of response. No patient was known to be resistant to doxorubicin, since the patients who had previously received doxorubicin or doxorubicin-containing regimens had responded to the drug or drug regimen and subsequently relapsed when doxorubicin was discontinued. Informed consent was obtained from each patient

TABLE 1.—Doxorubicin-DNA phase I trial: clinical diagnosis and response

Diagnosis	No. of patients	No. of responses*				
		CR	PR	SD	PD	ED
Carcinoid tumor	4	1 (6)	1 (8)	1 (4)		1
Chondrosarcoma	1				1	
Leiomyosarcoma	2			1 (5)	1	
Nodular poorly differentiated lymphocytic lymphoma	2			2 (3,2+)		
Poorly differentiated malignant neoplasm	3			1 (3)	1	1
Carcinoma						
Gastric	3				3	
Thyroid	2			1 (4)	1	
Breast	3			1 (3+)	2	
Prostatic	1			1 (5+)		
Mediastinal choriocarcinoma (male)	1		1 (3)			
Renal	1			1 (3)		
Parotid acinar cell	2			1 (1)	1	
Hepatoma	1			1 (3)		
Lung						
Adenocarcinoma	5		1 (4)	1 (3+)	2	1
Small cell	1					1
Squamous cell	1			1 (7)		
Salivary gland adenoid	1			1 (3)		
Pancreas	1			1 (5)		
Total	35	1	3	15	12	4
% response in patients entered		3	9	43	34	11
% response in evaluable patients		3	9	48	39	
		12				
			60			

*Values in parentheses = response durations in mos. SD = stable disease, PD = progressive disease, ED = early death.

according to institutional policies, prior to initiation of the study.

The drug was administered at a rate of approximately 100 ml of doxorubicin-DNA complex solution per hour, necessitating a 4–6-hour infusion for an average dose. More rapid infusion resulted in severe chills and fever. Extreme care was taken to avoid extravasation. The drug was given iv at 3–4-week intervals, depending on myelosuppression. The dose ranged from 20 to 135 mg/m^2 (median, 60) with 73% of the doses between 60 and 90 mg/m^2. Starting doses of doxorubicin-DNA were based on the known toxic effects of free doxorubicin, and too few patients received doxorubicin-DNA doses < 60 or > 95 mg/m^2 to make analysis of these doses meaningful. The initial dose was generally 60–75 mg/m^2 depending on marrow reserve, with appropriate dose modifications made for abnormalities of liver function according to those routinely employed for doxorubicin alone (9), and subsequent doses were escalated or reduced depending on toxicity from the previous course. All drug doses in milligrams per square meter refer to doxorubicin concentration.

Complete response (CR) was defined as the disappearance of all clinical evidence of active tumor for a minimum of 4 weeks, together with the disappearance of all tumor-related symptoms. Partial remission (PR) was defined as a 50% decrease in the sum of the products of the largest perpendicular diameters of all measured lesions lasting at least 4 weeks, in the absence of significant increase in any one lesion or the appearance of new lesions. Stable disease included tumor regression less than a PR lasting a minimum of 4 weeks or a steady state of response less than progressive disease of at least 8 weeks' duration. Progressive disease was defined as an unequivocal increase of any measured lesion by 25% or the appearance of a new lesion or lesions. Cardiotoxicity was diagnosed clinically by congestive heart failure without another established etiology; electrocardiographically by a $> 30\%$ decrease in the sum of the QRS voltage in the six limb leads as described by Minow et al (10); and in selected patients by endomyocardial biopsy.

RESULTS

Toxicity

Nonhematologic toxicity was evaluable in 114 courses given to 35 patients (table 2). As mentioned previously, doses of doxorubicin-DNA complex ranged from 20 to 115 mg/m^2 depending on marrow reserve and liver function. Fever and chills was a frequently mentioned side effect and was most prominent when the drug was administered rapidly. Slowing of the infusion rate to ≤ 100 ml/hour and premedication with antihistamines abolished or reduced these side effects so that subsequent doses could be given without their recurrence. Nausea and vomiting was noted in 11 patients and, although not reported, may have occurred in more. Nausea and vomiting cleared rapidly within 24 hours in most patients and was never dose-limiting. Alopecia was universal in patients receiving multiple courses of the drug. Mucositis was seen in six patients; it was severe in two. There were nine episodes of fever, with infection documented in three. The infections included two episodes of pneumonia and one of septicemia. There were nine episodes of fever of unknown origin, which resolved during antibiotic therapy. All episodes of fever and infection occurred while patients were neutropenic ($<$ 1000/mm^3 absolute granulocytes). There were two possible drug-related deaths: one from progressive pneumonia that was unresponsive to antibiotic and one due to an acute pulmonary hemorrhage in a patient with a normal platelet count. The latter patient had presump-

TABLE 2.—Nonhematologic toxicity of doxorubicin-DNA in 35 patients*

Toxic effect	No. of episodes in 114 courses (%)	No. of patients† (%)
Nausea and vomiting	15 (13)	11 (32)
Hives	2 (2)	2 (6)
Fever and chills (related to drug administration)	8 (7)	8 (24)
Alopecia	—	(100)
Cardiac toxicity ($>$ 30% decrease in QRS voltage)	—	6 (18)
Mucositis	6 (5)	6 (18)
Diarrhea	1 (1)	1 (3)
Extravasation	2 (2)	2 (6)
Documented infection	2 (2)	3 (9)
Fever of unknown origin	9 (8)	9 (26)
Drug-related death	—	2 (6)

*Dose range, 20–135 mg/m^2 (median, 60).

†Alopecia was universal in patients receiving multiple courses of therapy.

tive evidence of tumor involvement in the mediastinum and died unexpectedly 7 days after drug administration. Tumor necrosis or progression may have caused this fatal hemorrhage. No evidence of hepatic or renal drug toxicity was encountered in the study. Because of the paucity of patients treated at variable doses, no definite relationship between dose and nonhematologic toxicity can be determined.

Hematologic toxicity in this phase I study could only be evaluated at three dose levels: 60, 75, and 90 mg/m^2 (table 3). There were too few courses at other doses to make an analysis possible. Patients treated at these doses had grossly normal liver function tests and blood cell counts recorded at least weekly and frequently twice weekly. The major myelotoxic effects were leukopenia and granulocytopenia. The median nadirs were seen on Day 14; the median wbc count nadirs were 2300 and 1500/µl and the median granulocyte count nadirs were 800 and 320/µl for the 60- and 75-mg/m^2 doses, respectively. The median platelet count nadir occurred on Days 12 and 13, with counts of 131,000 and 155,000/µl for the 60- and 75-mg/m^2 doses. Granulocyte recovery was complete and a second cycle of chemotherapy was administered 23–24 days (median) after the first course. There was no evidence of cumulative myelosuppression with repeated courses of therapy.

Cardiac abnormalities were seen in two patients. The first patient had received prior doxorubicin, cyclophosphamide, and chest radiotherapy before doxorubicin-DNA therapy. After a dose of 150 mg/m^2 the ECG voltage decreased about 30%. The patient died shortly after this from infectious complications. At autopsy, there was evidence of arteriosclerotic cardiovascular disease but no evidence of doxorubicin cardiomyopathy. The second patient was elderly and had pre-existing heart disease; he had not received prior doxorubicin chemotherapy. However, after 290 mg/m^2 of doxorubicin-DNA complex, he developed a 35% decrease in QRS voltage. The patient was known to have pre-existing heart disease and cardiac enlargement, which had not changed. Cardiac function studies were attempted without success, and he was treated with digoxin. The patient's neoplasm had just begun to progress at that time and the drug was discontinued.

Response

Of the 35 patients entered in the study, four died within 3 weeks of entry from progressive tumor or complications related to their disease or chemotherapy. There were 31 patients, therefore, who were considered to be evaluable for tumor response (table 1). In these patients, there was one CR (3%) lasting 6+ months, and there were three PRs (9%) lasting 8, 4, and 3 months. Fifteen patients (48%) had stable disease. Five of those patients (16%) showed tumor regression less than PR lasting 7+, 4, 3+, and 2 months. The other ten patients had disease stabilization for a median of 3 months (range, 2–5+). Twelve patients (39%) had progressive disease. Two patients with carcinoid tumors responded to doxorubicin-DNA (one CR, one PR), as did a male patient with choriocarcinoma (PR) and a female with lung adenocarcinoma (PR). Disease stabilization or improvement was noted in other doxorubicin-responsive tumors (table 1). None of the responders (CR + PR) and only one of the patients with tumor regression less than PR had received prior doxorubicin. This patient was known to have doxorubicin-sensitive disease and was given doxorubicin-DNA after 500 mg/m^2 of doxorubicin, with continued improvement of her neoplastic process.

DISCUSSION

The response rate of 12% (CR + PR) in the phase I study is less than expected from patients who had received no prior treatment with doxorubicin. However, 12 of our patients had received prior doxorubicin, and a majority had received multiple prior chemotherapeutic regimens (median, three). Sixty-three percent of the patients showed tumor regression or disease stabilization, suggesting definite drug activity. Two of the four responses occurred in patients with carcinoid tumor, a malignancy recently reported to be responsive to doxorubicin therapy (11). There was a paucity of

TABLE 3.—Doxorubicin-DNA: hematologic toxicity[*]

Dose (mg/m^2)	No. of courses	Wbc count nadir, × 10^3 cells/µl	Granulocyte count nadir, × 10^3 cells/µl	Day of nadir	Platelet count nadir, × 10^3 cells/µl	Day of nadir	Day of next course of therapy[†]
60	35				131 (7–410)	13 (8–19)	23 (19–33)
	37	2.3 (0.6–4.7)	0.80 (0–2.6)	14 (4–23)			23 (19–33)
75	20	1.5 (0.6–13.1)	0.32 (0–10.0)	14 (11–20)	155 (16–384)	12 (8–16)	24 (14–34)
90	7	2.4 (0.1–4.0)	1.10 (0–21.0)	14 (7–20)	111 (66–292)	9 (6–15)	21 (19–30)

[*] Values are medians, with ranges in parentheses.

[†] Granulocyte count > 1.5 × 10^3 cells/µl; platelet count > 100 × 10^3 cells/µl.

patients in this study, previously treated or untreated, with more common tumors that are doxorubicin-responsive (lymphomas, sarcomas, small cell bronchogenic carcinoma, and carcinoma of the breast), since they receive doxorubicin in primary combination therapy in our department. Thus, an accurate comparison of the response rates in this study with those expected for free doxorubicin is not possible.

Because of prior studies on the acute toxic effects of doxorubicin (12), we began our phase I study of doxorubicin-DNA at comparable doses. This allowed us to rapidly determine the maximum tolerated dose in a small number of patients. Dose-limiting toxicity was myelosuppression, particularly granulocytopenia, subjecting patients to increased risk from infection. Considering that 84% of the patients had received extensive prior chemotherapy and that 37% had received prior radiation therapy, the leukopenia seen was similar to that for doxorubicin given by intermittent infusion at comparable doses.

The incidence of mucositis in our study (18% of the patients) was slightly higher than that seen with intermittent, high-dose, single-dose doxorubicin (9). Two episodes of severe mucositis were seen in one patient who had abnormalities of liver function tests and one who was given a dose of 90 mg/m^2. In a preliminary phase I study with doxorubicin-DNA complex (75 mg/m^2) in 12 patients, Rozencweig et al (13) found a slightly greater incidence (30%) of mucositis. The incidence and severity of most other acute toxic effects in our study were similar to those found by Rozencweig et al and those seen with free doxorubicin.

The incidence of hives and fever and chills was significant in the early studies and appeared to be clinically similar to a histamine-release phenomenon. This was definitely related to rapid drug infusion and was perhaps a reaction to the DNA. The reaction was largely eliminated by slowing the rate of infusion to < 100 ml/hour and premedicating the patients with antihistamines. Despite the inconvenience of prolonged infusions and the increased risk of extravasation that accompanies it, we cannot recommend more rapid drug administration.

The major hope of the lysosomotropic drug is to eliminate or reduce cardiac toxicity attributed to doxorubicin. Unfortunately, our patients did not receive enough cumulative doses of the drug to allow us to determine if there is decreased doxorubicin cardiotoxicity when the drug is bound to DNA. This was true of the patients of Rozencweig et al as well.

In this study, we were able to determine that a dose of 50–60 mg/m^2 of doxorubicin-DNA every 3 weeks had tolerable toxicity similar to that of doxorubicin.

Based on the results of these studies, we conclude that doxorubicin-DNA has significant antitumor activity and we currently recommend a starting dose of 50 mg/m^2 in patients with compromised bone marrow reserve and a starting dose of 60 mg/m^2 in patients with adequate bone marrow reserve and normal liver function. The drug can be repeated at 3–4-week intervals depending on myelotoxicity and may be escalated approximately 25% if there is no evidence of drug toxicity. Appropriate dose modifications should be made for liver dysfunction or when the drug is used with other myelosuppressive drugs. The major clinical toxic effects of doxorubicin-DNA are gastrointestinal and hematopoietic, with dose-limiting granulocytopenia.

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Phase I Study of SOAz[1,2]

S. Nasca, D. Jezekova, P. Coninx,* E. Garbe, Y. Carpentier, and A. Cattan[3,4]

SOAz, an inorganic cyclic derivative, was given as a single iv injection through one cycle in a phase I trial. Dose-limiting toxic effects noted were neutropenia and thrombocytopenia occurring at doses $\geqslant$ 220 mg/m^2 in patients with prior myelosuppressive treatment. The time to nadir for blood cell counts was long, as well as the time to recovery, so cumulative toxicity could be suspected. No other significant toxicity was noted. In patients with measurable tumor volume, no significant antitumor responses were seen. For further study, the dose of 220 mg/m^2 every 6–8 weeks according to hematologic restoration is recommended. [Cancer Treat Rep 66:2039–2042, 1982]

SOAz (1), an inorganic cyclic derivative, is a relative of MYKO 63 (hexaziridinocyclotriphosphazene) (2). This latter drug was successfully tested on several experimental tumors (3), but its therapeutic index was disappointing because of cumulative toxicity. Chemists therefore studied other molecules and synthesized SOAz (pentaziridinocyclodiphosphathiazene; fig 1) (4,5). Animal toxicity trials of this drug were conducted in mice, rats, beagle dogs, and monkeys.[5] The most prominent finding was hematologic toxicity with leukopenia and thrombocytopenia but without cumulative effect. The activity of SOAz was described in vitro against various tumors of human or animal origin including S lymphoma, R lymphoma, lymphosarcoma, neuroblastoma, rhabdomyosarcoma, fibroblastoma, ovarian carcinoma, HeLa cervix carcinoma, Ehrlich ascites carcinoma, osteosarcoma, subcutaneous melanoma, and Lewis lung carcinoma (6). Activity of SOAz was reported against several rodent tumors in vivo, including P388 and L1210 leukemias, Lewis lung carcinoma, subcutaneous B16 melanoma, Sarcoma 180, and Y. M. 12 sarcoma (4,7).[5] Furthermore, encouraging therapeutic results were obtained in spontaneous osteogenic sarcoma of the dog without any hematologic toxicity. Although the mechanism of action of SOAz was unknown, this drug was selected for a phase I study on the basis of a possible high therapeutic index. The trial was designed to determine the maximum tolerated dose (MTD) for a single iv injection.

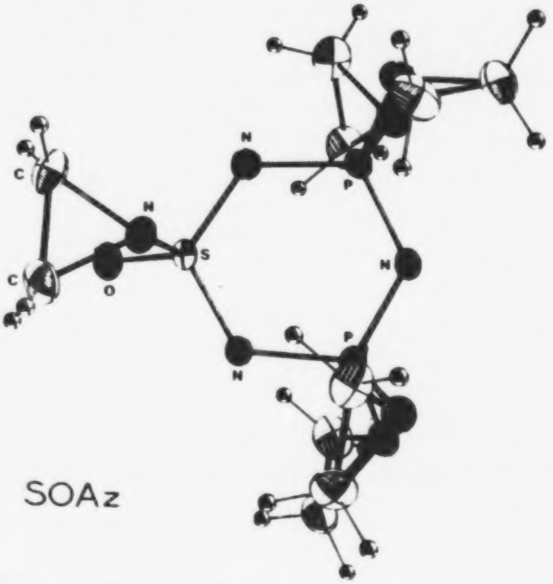

FIGURE 1.—Compound structure with conventional chemical symbols.

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[1] Received May 17, 1982; revised Sept 14, 1982; accepted Sept 28, 1982.

[2] Supported in part by grants from CNRS-PIRMED and ARERS.

[3] Institut Jean Godinot, Reims, France.

[4] We thank H. Roelandt and C. Galdies for assisting in this study.

[5] Unpublished results from Otsuka Chemical Co, Ltd, Osaka, Japan.

*Reprint requests to: P. Coninx, MD, Department of Medicine, Institut Jean Godinot, B. P. 171, 1, Avenue Général Koenig, 51056 Reims Cedex, France.

PATIENT SELECTION

Patients with a histologically confirmed diagnosis of malignancy were eligible for this study regardless of age, tumor site, and previous therapy, provided that the tumors were refractory to conventional modalities of treatment or that no effective therapy was known. Informed consent from all patients was obtained and none were given radiotherapy or chemotherapy within the 4 weeks prior to SOAz administration. Criteria for entry in this study included a performance status ≥ 40 (Karnofsky scale) (8), an expected survival time of at least 8 weeks, an adequate hematologic status (granulocyte count ≥ 2000/µl and platelet count ≥ 100,000/µl), an acceptable renal creatinine (≤ 1.7 mg/100 ml), and acceptable hepatic function (bilirubin and transaminases < 1.5 times normal value).

TREATMENT SCHEDULE

SOAz was supplied as a dry powder without excipients by the Otsuka Chemical Co, Ltd. SOAz vials (300 mg) were reconstituted in 5 ml of sterile normal saline. All doses were administered as a 5-minute rapid infusion through a free-flowing iv line. The starting dose was 65 mg/m^2, based on $^1/_3$ of the minimal toxic dose in the most sensitive animal species (dog), and dose escalation (table 1) was carried out according to Fibonacci's search scheme (9). Each patient was given a single dose through one cycle; at least three patients were treated at each dose level and were observed until recovery occurred before other patients were entered at a higher dose level. As soon as the MTD was determined, other patients were entered at lower dose levels (six patients) or at the MTD level (12 patients) (table 1) so that the toxic manifestation could be studied accurately.

STUDY PARAMETERS

Pretreatment assessment included a complete history and physical examination with documentation of metastatic disease and objective tumor measurement when possible. Laboratory study included a cbc with differential and platelet count, chemistry panel (BUN, creatinine, electrolytes, glucose, total protein, albumin, total bilirubin, SGOT, SGPT, alkaline phosphatase, and fibrinogen), urinalysis, ECG, and chest roentgenogram. Follow-up included weekly cbc with differential and platelet count; chemistry panel, urinalysis, and ECG were checked at 1- and 3-week intervals. Repeated physical examinations with tumor measurement were performed at 3-week intervals. All the investigations included in patient assessment were carried out again at the time when recovery from toxic effects occurred.

TABLE 1.—Relationship between dose and No. of patients

Dose (mg/m^2)	No. of patients	
	Total	Men/women
65	6	3/3
133	6	3/3
220	12	8/4
330	3	3/0
467	2	2/0

When we became aware of the long period for hematologic recovery, we elected to study eight patients, the last to be entered in the trial, to determine the blood cell kinetics with higher accuracy at MTD. Therefore, a weekly follow-up through a period of 8 weeks was carried out. Toxicity was defined by standard World Health Organization (WHO) criteria (10).

RESULTS

Patient characteristics are detailed in table 2. One of the patients, who had been erroneously selected for this study, could not be evaluated because he died from progressive disease soon after SOAz administration. The number of individuals treated at each dose level is

TABLE 2.—Patient characteristics

Characteristic	No. of patients
Total	30
Evaluable	29
Sex	
Men	19
Women	10
Median age in yrs (range): 69 (27–81)	
Previous treatment	
Chemotherapy only	5
Radiotherapy only	10
Chemotherapy and radiotherapy	12
Disease	
Squamous cell carcinoma	
Head and neck	8
Cervix	3
Anus	1
Esophagus	1
Bronchus	1
Basal cell carcinoma	
Skin	1
Adenocarcinoma	
Colorectum	4
Prostate	2
Stomach	1
Kidney	1
Breast	1
Endometrium	1
Unknown	2
Sarcoma	
Soft tissue	1
Osteosarcoma	1

TABLE 3.—Myelosuppressive toxicity: median nadir blood cell count and median day of nadir

Dose (mg/m²)	Polymorphonuclear cells		Mononuclear cells		Platelets	
	Median count* (range)	Median day (range)	Median count* (range)	Median day (range)	Median count* (range)	Median day (range)
65	3.1 (2.5–5.2)	21 (8–22)	0.5 (0.4–1.1)	19 (7–25)	163 (61–207)	22 (14–31)
133	3.1 (0.8–4.5)	19 (7–35)	0.5 (0.1–1.4)	17 (7–35)	158 (22–218)	24 (15–43)
220	1.7 (0.3–4.0)	30 (15–43)	0.5 (0.1–0.8)	28 (18–36)	74 (11–195)	28 (18–36)
330	0.6 (0.4–1.1)	28 (27–29)	0.1 (0.1–0.2)	29 (21–35)	48 (32–55)	23 (21–28)
467	0.1 (0.1–0.2)	25 (22–27)	0.1 (0.1–0.1)	25 (22–27)	31 (25–37)	21 (21–22)

* × 10^3 cells/µl.

shown in table 1. No patient receiving SOAz showed an unacceptable hepatic function, whereas the creatinine levels were high in four patients: a dose of 65 mg/m² (creatinine, 1.5 and 1.6 mg/100 ml), two patients; 133 mg/m² (creatinine, 1.5 mg/100 ml), one; and 220 mg/m² (creatinine, 1.7 mg/100 ml), one. Hematologic toxicity was severe in some cases but never lethal. For the four patients with a high creatinine level, the bone marrow depletion did not seem to be more severe. As shown in table 3, neutropenia and thrombocytopenia were dose-related and became dose-limiting at 220–330 mg/m². Platelet variations after SOAz administration at a dose of 330 mg/m² are documented in figure 2. The same pattern was observed for polymorphonuclear cells, with cell count nadir at Week 4 and recovery at Weeks 6–7. Blood transfusions were required for six patients who were given SOAz at a dose level > 65 mg/m²: one at 133 mg/m², four at 220 mg/m², and one at 467 mg/m². For these patients the median fall in hemoglobin was 4.6 g/100 ml (range, 3.1–6.5) versus 2.3 g/100 ml (range, 0.31–3.7) for the others who did not receive blood transfusions. The median day of the erythrocyte count nadir was Day 35 (range, 15–56).

In the eight patients who were given SOAz at a dose of 220 mg/m² and who had had weekly wbc counts for 8 weeks, the median recovery times were 50 days (range, 38–60) for polymorphonuclear cells and 46 days (range, 38–50) for platelets. Of these patients, four had to be given blood transfusions because their hemoglobin was < 7.5 g/100 ml. Furthermore, it appeared that the more extensive prior myelosuppressive treatment was, the deeper the nadir cell counts, the sooner their occur-

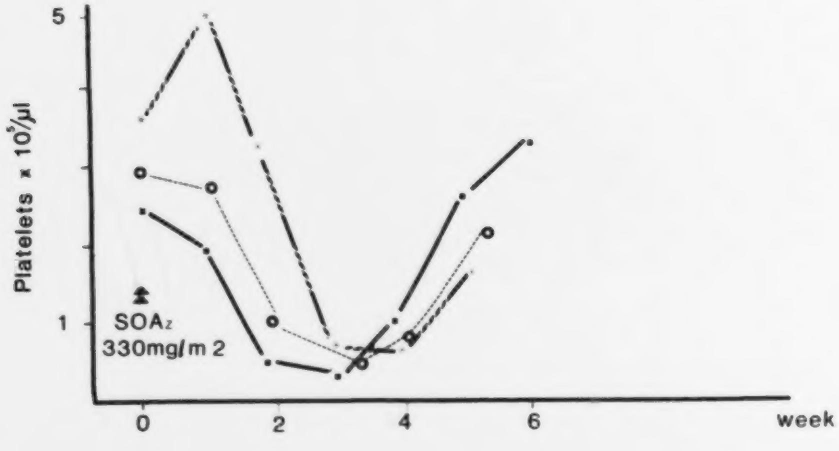

FIGURE 2.—Platelet counts after iv injection of 330 mg/m² of SOAz in 3 patients.

rences, and the later the recoveries.

Other toxic effects may be considered as negligible since they occurred infrequently and were of low grade (1 or 2) according to WHO criteria. Fever was observed in six patients, nausea and vomiting in two, and diarrhea in one. Immediately after infusion, two male patients also reported a moderately painful, strangling sensation in the chest without any symptoms or signs of heart disease. Another patient reported a fleeting sensation of mint breath during infusion.

The antitumor effect could be assessed in 24 patients. There were no complete or partial responders according to WHO criteria (10). Six patients achieved disease stabilization and 18 showed tumor progression.

DISCUSSION

The MTD of SOAz as a single dose is 220 mg/m^2 for patients with extensive prior myelosuppressive treatment. Neutropenia and thrombocytopenia, which were the main toxic effects, occurred almost simultaneously and recovery times were similar. These hematologic toxic effects were delayed, so the treatment could be repeated only about 40–60 days after the first administration. Another trial is in progress to determine a possible cumulative effect. Other manifestations of drug toxicity were negligible.

Although SOAz did not seem to be effective in this phase I study, the lack of antitumor activity should not preclude the initiation of phase II trials with this compound, but only after cumulative toxicity has been studied. The assessment of SOAz activity was limited in this study by the structure of the phase I trial; patients with extensive prior chemotherapy or radiotherapy were selected and low doses of SOAz were required. For further trials with this compound given as a single iv injection, we suggest the dose of 220 mg/m^2 every 6–8 weeks (according to hematologic restoration) for patients with prior myelosuppressive treatment, and we cautiously propose 330 mg/m^2 every 6–8 weeks for the patients who are not given such prior therapy.

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Medroxyprogesterone Acetate (MAP) Relative Bioavailability After Single High-Dose Administration in Cancer Patients[1]

Franco Pannuti,[2,*] Carlo M. Camaggi,[3] Elena Strocchi,[4] Michele Giovannini,[2] Anna R. Di Marco,[4] and Barbara Costanti[4]

A pharmacokinetic evaluation of medroxyprogesterone acetate (MAP; 6α-methyl-17α-hydroxyprogesterone acetate) was undertaken in 70 patients with advanced cancer treated either orally or im with single high doses. MAP plasma levels were determined by gas chromatography (^{63}Ni electron capture detector) after derivatization with heptafluorobutyric anhydride, using 17α-hydroxyprogesterone caproate as internal standard. Plasma levels after oral administration can be approximated by a triexponential function in agreement with a pharmacokinetic two-compartment open model with first-order absorption; peak levels are dose-related, but high interindividual variance is present. Following im administration, both decay phases are masked by prolonged absorption; peak levels are lower than after oral administration, but long-term bioavailability is higher, as demonstrated by comparison of the values for areas under curves in the 0–146-hour interval. The high interindividual ranges in the observed MAP plasma levels indicate that if any significant clinical evaluation is to be made, routine analysis of plasma MAP is mandatory. [Cancer Treat Rep 66:2043–2049, 1982]

Medroxyprogesterone acetate (MAP; 6α-methyl-17α-hydroxyprogesterone acetate) is a well-known synthetic progestogen whose action against hormone-dependent tumors has been recognized since the early 1960s. Only recently, however, extensive clinical trials have clearly pointed out that its effectiveness in advanced breast cancer therapy is greatly enhanced if it is used in high daily doses (500–2000 mg) (1–6). This behavior parallels the findings of in vitro studies, showing that MAP nearly inhibits the growth of the MCF-7 human breast cancer tumor cell line at 1.10^{-6} M concentration, whereas it is ineffective at the lower 1.10^{-8} M level (7). In addition, high-dose MAP ($\geq$ 500 mg/day) was found to be useful in the therapy of renal, prostatic, and endometrial tumors as well as in oncologic pain control (8–10).

After either oral or im administration, MAP is absorbed but no extensive bioavailability studies on this drug at different doses are reported. We now present the results of our study in which plasma levels of MAP were determined by gas chromatography in 70 cancer patients after oral or im administration of a single high dose.

MATERIALS AND METHODS

Chemicals (highest analytic grade) were supplied by E. Merck (Darmastadt). MAP (Farmitalia, Milan) and 17α-hydroxyprogesterone caproate (Shering; internal standard) were recrystalized several times until chromatographic purity. Chromatographic analyses were carried out on a Varian 3700 automatic gas chromatograph equipped with a ^{63}Ni electron capture detector, autosampler model 8000, and CDS 111 data system.

Statistical analysis of the results was accomplished with the SPSS program package (11). Nonlinear least-squares fit of the plasma decay of MAP concentrations was obtained using the Gauss-Newton algorithm as programmed in the BMDP package (12).

Patients entered in this study had histologically proved advanced cancer and were assessed as having normal liver and renal functions by standard clinical tests. All had received prior chemotherapy or radiation therapy, but oncologic treatments were discontinued at least 1 month before (and any other treatment at least 2 days before) the time of this study. Life expectancy was ≥ 2 months.

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[1] Received Aug 5, 1981; revised Sept 7, 1982; accepted Sept 21, 1982.

[2] Divisione di Oncologia, Ospedale M. Malpighi, Bologna, Italy.

[3] Istituto di Chimica Organica, Università degli Studi Viale Risorgimento, Bologna, Italy.

[4] Associazione Nazionale per lo Studio e la Cura dei Tumori Solidi, Divisione di Oncologia, Ospedale M. Malpighi, Bologna, Italy.

[*] *Reprint requests to:* Prof. Franco Pannuti, Divisione di Oncologia, Ospedale M. Malpighi, 15 Bologna, Italy.

Each patient received a single dose of MAP either orally (500, 1000, 2000, or 3000 mg) or im (500, 1000, or 2000 mg) (Farlutal depot vials; ten patients for each treatment). Oral doses were administered in fruit juice. Blood samples were collected before and at various times after MAP administration (1, 2, 4, 6, 8, 10, 26, 30, 50, 74, 98, and 146 hours) up to the seventh day. In all experiments venous blood samples were taken in heparinized tubes and centrifuged. Plasma samples were kept frozen at -20°C until analysis.

Extraction and Derivatization Procedure

As previously proposed by Kaiser and co-workers (13), MAP was analyzed after derivatization with heptafluorobutyric anhydride as 3-enol heptafluorobutyrate. A 1-ml plasma sample, 100 µl of a stock solution (containing about 200 ng of 17α-hydroxyprogesterone caproate as internal standard), and 1 ml of distilled water were combined in an 18 × 100-mm silanized glass centrifuge tube. This mixture was extracted twice in a horizontal shaker with 3 + 3 ml of cyclohexane. The organic phase was then completely evaporated under nitrogen at 30°C, leaving a residue which was then directly treated with 1 ml of acetone:acetonitrile (1:1) plus 50 µl of heptafluorobutyric anhydride. After 1 hour at 30°C, this solution was evaporated under nitrogen without further heating. The residue was reconstituted with 1 ml of cyclohexane in a vortex mixer and transferred in the standard minivial of the chromatograph's autosampler programmed for 1-µl injections.

Chromatographic Analysis

Several stationary phases and conditioning of the chromatographic column were tested in trial runs. Considerable separation of the 3-enol ester of MAP and internal standard from the other components of the injected mixture was achieved with several commercially available OV-1 or OV-17 silicones on silanized Chromosorb W glass columns; but an accurate reproduction of the analytic results was obtained only after using 3% Supelco SP-2250 on 100–120 Supelcoport. The analytic method was calibrated using blank plasma samples (1 ml) spiked with various amounts of MAP and 17α-hydroxyprogesterone caproate (internal standard). The typical run-to-run coefficient of variation was < 8% for plasma levels > 2 ng/ml. No interferences from endogenous or exogenous materials were observed; however, since MAP is a highly stable and insoluble compound, extreme care is necessary in cleaning all the glassware (chromic acid), and plasma samples must be collected with disposable syringes to avoid contamination problems.

The absolute minimal detectable quantity of MAP is about 0.5 pg per chromatographic injection; however, the accuracy of the method decreases for plasma levels < 2 ng/ml, because of cleanup problems. The detector response is linear in the 1–500-pg range (corresponding to plasma levels of 1–500 ng/ml).

All of the routine analyses were carried out with the following chromatographic conditions: 2 m × 3 mm (inside diameter) silanized column (3% SP-2250 on 100–120 Supelcoport, preconditioned for 1 week at 340°C); injection port temperature, 270°C; detector temperature, 300°C; column oven temperature program, 230°C to 270°C at 2°C/minute; carrier gas (nitrogen), 30 ml/minute; and injections of 1 µl. A blank plasma sample was included with every batch processed. Calibration runs were carried out weekly without detection of noticeable changes in the relative response factor.

A typical gas chromatographic analysis is shown in figure 1.

RESULTS

Mean plasma levels of MAP following single-dose administration are reported in semilog plots in figures 2 and 3, where each value is the mean of results from the ten patients receiving each dose. The individual time/concentration profile of each patient shows some discontinuities, but can be interpolated by a triexponential function (14).

$$C = -A.e^{\alpha t} + B.e^{\beta t} + C.e^{\gamma t}.$$

The half-life parameters obtained by the best-fit procedure are reported in table 1. After oral administration, plasma levels rapidly increase, reaching a peak after 1–2 hours (fig 2), followed by a biexponential decay. The decaying process involves an initial half-life of about 1.4 hours and a terminal-phase half-life of about 58.8 hours. However, while the determination of first half-life is sufficiently accurate, the second half-life suffers some inaccuracy, since analysis of plasma levels is less reliable at < 2 ng/ml. At 1–2 hours after the im administration, a steady plasma level is reached and thereafter maintained for the duration of the 7 days (fig 3). Figure 4 shows a scattergram of the correlation between the plasma MAP peak levels observed in 40 patients who received various oral doses.

Linear regression analysis of these data shows a linearity, in the range of 1000–3000 mg, between peak MAP levels and administered dose. The ANOVA (analysis of variance) relative to both the regression procedure and pure error indicates the significance of the linear regression (99% level) (15). A similar procedure

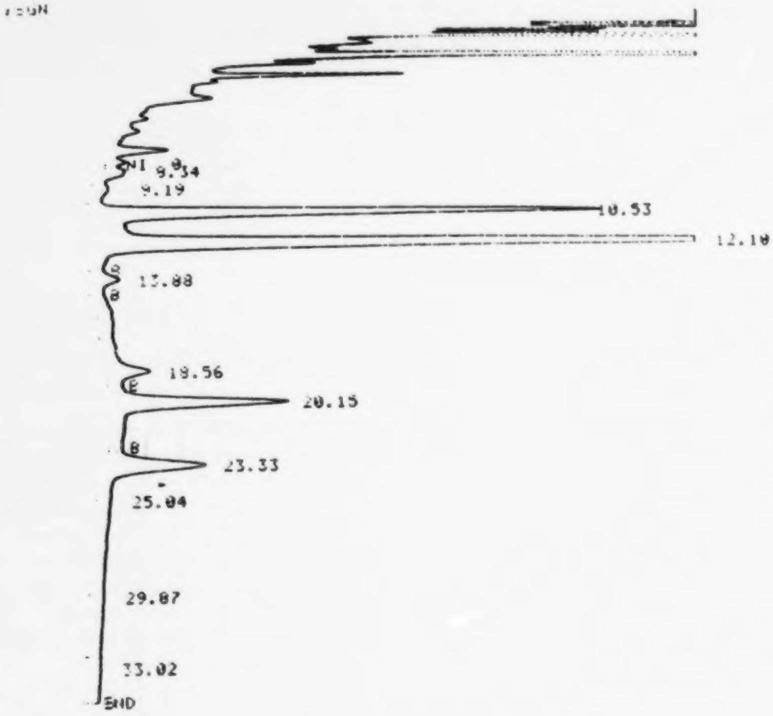

FIGURE 1.—Gas chromatogram showing a typical analysis: 500 mg, im, 26 hrs: MAP level, 5.3 ng/ml (retention time, 13.88 mins); 17α-hydroxyprogesterone caproate (internal standard), 134.2 ng/ml (retention time, 20.15 mins).

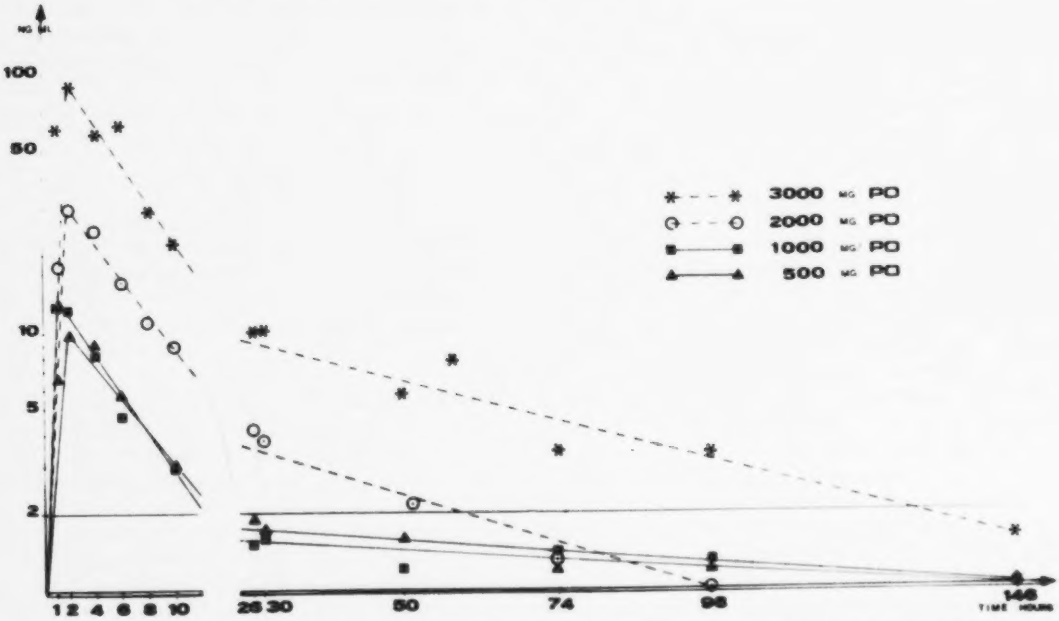

FIGURE 2.—Plasma MAP levels following oral administration. Accuracy of determining MAP levels < 2 ng/ml is low.

TABLE 1.—MAP interpolation of absorption/decay curves (oral administration)*

Parameters	MAP dose							
	500 mg		1000 mg		2000 mg		3000 mg	
	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
α	–0.77	0.52	–0.88	0.16	–0.64	0.28	0.80	0.33
$t_{1/2}\alpha$	1.05	0.45	0.81	0.16	1.30	0.60	1.00	0.40
β	–0.52	0.14	–0.61	0.22	–0.51	0.17	0.42	0.15
$t_{1/2}\beta$	1.43	0.37	1.25	0.39	1.29	0.26	1.63	0.39
γ	†	†	†	†	–0.015	0.006	–0.011	0.006
$t_{1/2}\gamma$	†	†	†	†	53.2	15.2	64.5	28.6

*$\bar{X}$ = mean of data from individual patients; $t_{1/2}$ = hours.

†Because of the low accuracy of the analytic method at < 2 ng/ml, these data are scarcely significant.

applied to plasma levels after im administration reveals less significant statistical correlation.

The area under the concentration/time curve was calculated for individual patients using the trapezoidal rule in the 0–146-hour time interval. The results (tables 2,3) are in agreement with the data obtained by analytic integration of the interpolated curves in the same time span. Here again, the existence of a statistically significant linear correlation between administered dose and area under the concentration/time curve (0–146 hours) was tested by analysis of variance; in this case a correlation ($P < 0.001$) was found for both oral and im administration.

DISCUSSION

Although the intrinsic low specificity of the radioimmunoassay (RIA) of MAP can now be partially overcome by using a selective extraction technique, we have chosen to use a gas chromatographic method, which assures the complete absence of interferences with MAP metabolites and offers a more accurate run-to-run coefficient of variation and a higher linearity range.

It is of interest to compare the decay curves of MAP plasma concentrations after oral administration determined by gas chromatographic analysis with those reported using RIA techniques (16). In the latter case, peak plasma levels are shifted at longer times after administration, and fairly high amounts of MAP are still observed after 168 hours.

These apparent discrepancies from our data can probably be explained by cross-reactivities with MAP metabolites in the RIA procedure (17). The simplest pharmacokinetic model consistent with the observed plasma decay after oral administration is a two-compartment open model. The first decay phase (table 1) can probably be attributed to the distribution in peripheral tissues and is obscured after im administration as a result of the prolonged absorption. The subsequent "biologic half-life" is considerably longer. Although the

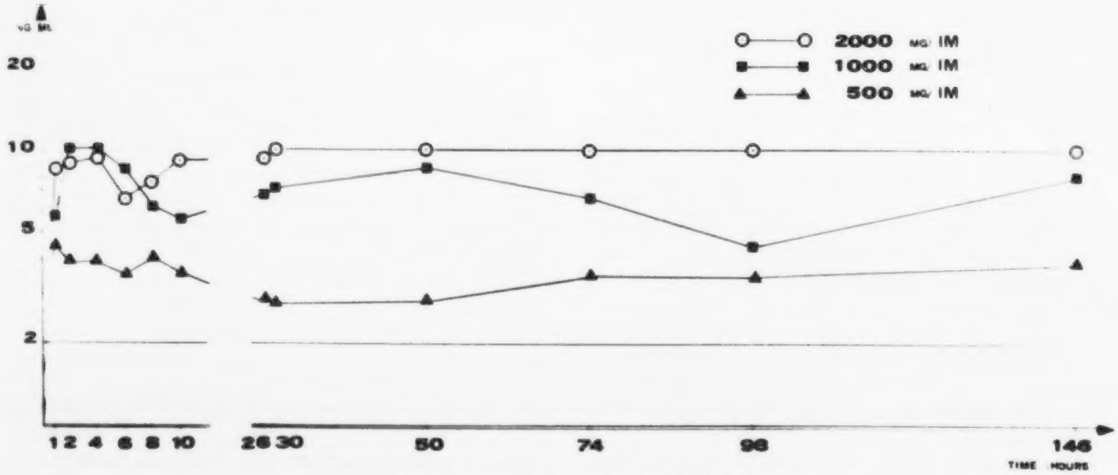

FIGURE 3.—Plasma MAP levels following im administration.

TABLE 2.—**MAP areas under absorption/decay curves (ng.h.ml^{-1}) of individual patients (oral administration)**

	ng.h.ml^{-1} for MAP dose—			
	500 mg	1000 mg	2000 mg	3000 mg
	126.5	630.0	454.8	229.3
	227.4	381.5	894.4	653.6
	291.6	175.5	382.0	1123.1
	64.3	181.9	587.3	3217.2
	256.9	240.0	400.1	1931.7
	336.2	255.4	141.5	908.7
	336.2	248.0	985.2	829.6
	409.0	160.6	358.6	910.0
	251.9	13.7	296.6	1605.0
	309.9	46 6	125.0	980.2
Mean value	255.0	233.2	442.6	1238.8
± SD	16.0	174.6	306.1	840.3

TABLE 3.—**MAP areas under absorption/decay curves (ng.h.ml^{-1}) of individual patients (im administration)**

	ng.h.ml^{-1} for MAP dose—		
	500 mg	1000 mg	2000 mg
	404.0	1648.3	668.5
	358.1	2010.7	3960.5
	398.2	1893.4	1096.4
	173.1	1174.3	2702.6
	669.4	386.2	1435.7
	250.5	474.1	1248.9
	470.7	881.6	536.1
	1423.8	299.0	1295.4
	422.8	294.2	638.5
	464.6	853.7	1451.5
Mean value	503.5	991.6	1503.0
± SD	349.4	662.3	1061.3

differences in these half-life parameters at different doses are not statistically significant, the best estimate of the biologic half-life of MAP is probably derived from the 3000-mg (oral administration) curve, which is less affected by the low accuracy of the analytic data < 2 ng/ml.

Some interesting conclusions can be drawn from the data reported in the previous section. MAP is rapidly absorbed after oral administration, and plasma concentration of the drug increases significantly with respective increases in the administered dose. Bioavailability is highly variable (tables 2 and 3 and fig 4), and this must be taken into account when treatment is scheduled. In addition, the high interindividual range in plasma levels indicates the need to improve the absorption efficiency of this drug, especially by testing new dosage forms that are better suited for high-dose oral treatment than the presently used vials optimized for im administration. Preliminary results in our laboratory show that by using 500-mg MAP tablets instead of aqueous suspension as a drug delivery system, both the mean plasma MAP concentrations and the bioavailability are higher. However, the large interindividual variance renders these differences scarcely significant. While im administration results in a better long-term absorption, the short-term bioavailability (0–24 hours) is more favorable after oral treatment.

The present findings have several important areas of clinical application. The knowledge of the dose-dependent MAP peak levels after oral administration, of the peculiar long-term absorption following an im treatment, and of the dose-dependent bioavailability could be relevant in planning any therapeutic use of this drug. In addition, the high interindividual variance in plasma

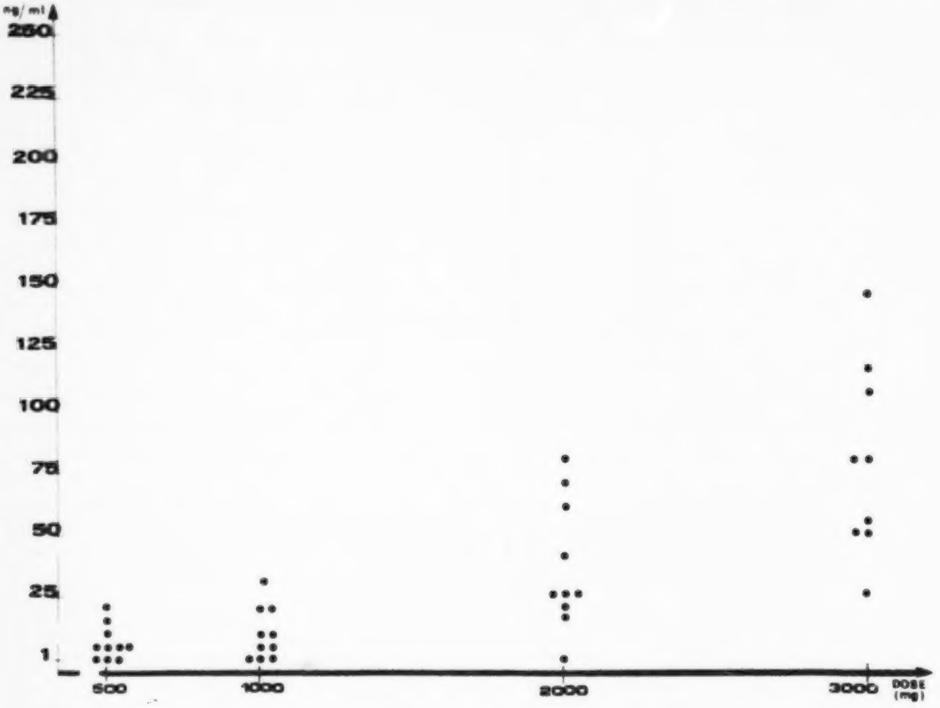

FIGURE 4.—Plasma MAP peak levels in individual patients after oral administration of different doses.

levels indicates that if any significant correlation with clinical response is to be made, the extent of MAP absorption must be determined for each patient.

In an ongoing study we are testing MAP plasma levels following multiple administration. The large intersubject spread in bioavailability is still present. Hopefully, more clinically significant conclusions will soon be reached.

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Comparison of Thymidine and Folinic Acid Protection From Methotrexate Toxicity in Human Lymphoid Cell Lines[1,2]

George P. Browman[3,*]

Hypoxanthine, thymidine, and folinic acid protection from methotrexate cytotoxicity were compared in human lymphoid cell lines variably sensitive to thymidine-induced growth suppression. Hypoxanthine protection differed among the cell lines, and the dose-response relationship for protection occurred within the physiologic bone marrow hypoxanthine concentration range. The slopes of the thymidine protection curves were virtually superimposable in a B cell line versus a T cell line, but threefold to fourfold higher thymidine concentrations were required to maximally protect the B versus the T cell line. Thymidine (plus hypoxanthine) protection was superior to that of folinic acid in both the B and the T cell lines when protection was delayed. As the interval between methotrexate and folinic acid exposure was progressively delayed, there was a linear decline in the degree of maximum protection in both cell lines. However, as thymidine exposure was progressively delayed, maximal protection was maintained, except at 12 hours in the B cell line. The influence of methotrexate on thymidine-induced growth suppression was studied. Methotrexate enhanced thymidine-induced growth suppression in sensitive cells as manifested by a significant shift of the dose-response curve. Deoxycytidine protection from thymidine toxicity was superior in the presence of methotrexate. The results of these studies indicate that thymidine protection and folinic acid protection against methotrexate toxicity produce different effects, which in part are dependent on the cell type. The complexity of these interactions points out the need for further studies. [Cancer Treat Rep 66:2051–2059, 1982]

Clinical studies have confirmed that thymidine infusions can protect against methotrexate toxicity in man (1–4). The use of thymidine with methotrexate provides the potential for differential selectivity of rescue between tumor and normal tissues, which could result in a superior antitumor effect. Studies in experimental systems show that protection from methotrexate toxicity requires a salvageable purine as well as thymidine. Therefore, clinical selectivity would depend on: (*a*) the physiologic purine levels, (*b*) the purine salvage capabilities of tumors and normal tissues, and (*c*) the thymidine salvage capabilities of tumors and normal tissues. In addition to determining differential rescue between normal and tumor tissues, these factors might also contribute to the antitumor spectrum of methotrexate.

Certain biochemical properties of human lymphoid cell lines suggest that thymidine utilization might vary among lymphoid subsets of B, T, and null cell origins. Cells with T and null cell characteristics are more sensitive to the growth-inhibitory effects of thymidine than are cells with B cell characteristics (5–8). This differential sensitivity seems to be related to differences of intracellular thymidine metabolism. Thymidine catabolism is more active in B cells, where thymidine phosphorylase levels or the ratio of thymidine phosphorylase to thymidine kinase is higher than in T and null cells (9,10). Furthermore, intracellular clearance of dTTP is more rapid in B cells exposed to high concentrations of thymidine (8,9). Deoxycytidine can reverse thymidine toxicity in vitro (11,12). Therefore, thymidine toxicity is thought to be due to allosteric inhibition of ribonucleotide reductase by excess dTTP, which results in

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[1] Received Feb 9, 1982; revised Sept 1, 1982; accepted Sept 21, 1982.
[2] Supported by the National Cancer Institute of Canada.
[3] Ontario Cancer Foundation, Hamilton, Canada.

**Reprint requests to:* George P. Browman, MD, Ontario Cancer Foundation, Hamilton Centre, 711 Concession St, Hamilton, Ontario L8V 1C3, Canada.

decreased conversion of CDP to dCDP (13). This will ultimately deplete intracellular dCTP levels and consequently DNA synthesis.

Methotrexate, a clinically useful drug for the treatment of a variety of lymphoid malignancies, produces a rapid depletion of intracellular dTTP pools (14). We therefore hypothesized that the enhanced thymidine catabolism and rapid clearance of dTTP by B cells might result in increased requirements for thymidine to protect against methotrexate toxicity. The in vitro studies reported here were designed to test thymidine requirements for methotrexate protection among lymphoid cell lines variably sensitive to thymidine-induced growth inhibition. Thymidine protection was compared to folinic acid protection, and we also examined the interaction of toxic concentrations of thymidine with methotrexate.

Since selective thymidine protection or rescue in vivo might also be influenced by ability to utilize circulating salvageable purines, we also studied hypoxanthine requirements for methotrexate protection among these cells.

MATERIALS AND METHODS

Human lymphoblastoid cell lines were provided by Dr. Herbert Lazarus of the Sidney Farber Cancer Institute (Brookline, MA) and Dr. Jun Minawada of Roswell Park Memorial Institute (Buffalo) and were maintained in suspension culture in RPMI 1640 tissue culture medium (Grand Island Biological Co [GIBCO], Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO), 20 mM HEPES buffer (Sigma Chemical Co, St. Louis), penicillin (100 units/ml) (GIBCO), and streptomycin (100 µg/ml) (GIBCO).

Methotrexate and folinic acid were obtained from Cyanamid Canada, Inc (Willowdale, Ontario), and thymidine, deoxycytidine, and hypoxanthine were obtained from Sigma Chemical Co. Thymidine requirements for methotrexate protection were assessed during a 72-hour growth period in which 2×10^5 cells in triplicate 1-ml cultures were exposed to a maximum inhibitory concentration of methotrexate (0.2–1.0 µM), a fixed concentration of hypoxanthine (80 µM), and varying concentrations of thymidine. The hypoxanthine concentration was determined in preliminary experiments, and hypoxanthine was added to cultures at the same time as methotrexate. Varying concentrations of thymidine were added to cultures under separate conditions, at the same time as methotrexate or at 1, 4, 6, or 12 hours after methotrexate to allow a progressive fall in dTTP levels. Hypoxanthine requirements for methotrexate protection were assessed in the same way as for thymidine except that the thymidine concentration was fixed and the hypoxanthine concentration was varied. Hypoxanthine protection was measured only with simultaneous exposure to methotrexate. For these experiments, medium was supplemented with extensively dialyzed fetal bovine serum. At the end of the incubation (72 hours after addition of thymidine or hypoxanthine), the cells were electronically counted in a Coulter Counter model ZF. Growth in treated cultures is expressed as a percent of the growth achieved in 72-hour control cultures without additives. The abilities of thymidine and hypoxanthine to protect against methotrexate, when the compounds were added simultaneously, is expressed as a PC_{50} value, which represents the concentration at which 50% of maximum protection is achieved, assuming zero protection as the effect of methotrexate alone.

Folinic acid protection was also measured to control for differences in thymidine protection between B and T cells that were unrelated to thymidine metabolism. For these experiments, the maximum protection provided by selected concentrations of folinic acid (described in Results section) was assessed 72 hours after folinic acid was added to methotrexate-treated cultures.

Thymidine toxicity was measured in the presence and absence of methotrexate by evaluating its growth-inhibitory effect at varying concentrations over a 72-hour exposure period. Growth inhibition is quantitatively expressed as an IC_{50} value, which corresponds to the thymidine concentration that inhibits growth to 50% of that of untreated cultures. Deoxycytidine reversal experiments were performed by adding the compound to cultures simultaneously with thymidine.

All cultures were documented to be free of mycoplasma contamination through serial determinations using the differential uridine:uracil incorporation method of Schneider et al (15) and the mycoplasma culture test kit provided by Flow Laboratories (McLean, VA). The growth rates of all cell lines were similar.

RESULTS

The maximum cytotoxic effect of methotrexate was reached in all cells at a concentration of 0.05 µM (data not shown). Simultaneous thymidine and hypoxanthine protection were assessed at a 1.0-µM concentration of methotrexate, while delayed protection with thymidine and folinic acid was assessed at a 0.2-µM methotrexate level.

Thymidine dose-response inhibition curves are depicted in figure 1. They confirm the enhanced thymidine sensitivities of the malignant T (CCRF-CEM and MOLT-4) and malignant null (LAZ 221) cell lines relative to the Epstein-Barr virus-transformed "normal" B cell line (LAZ 007). The differences in thymidine sensitivities are quantitatively expressed as IC_{50} values in the legend. Figure 2 shows the dose-response relationship for the protective effect of hypoxanthine in all the cell lines.

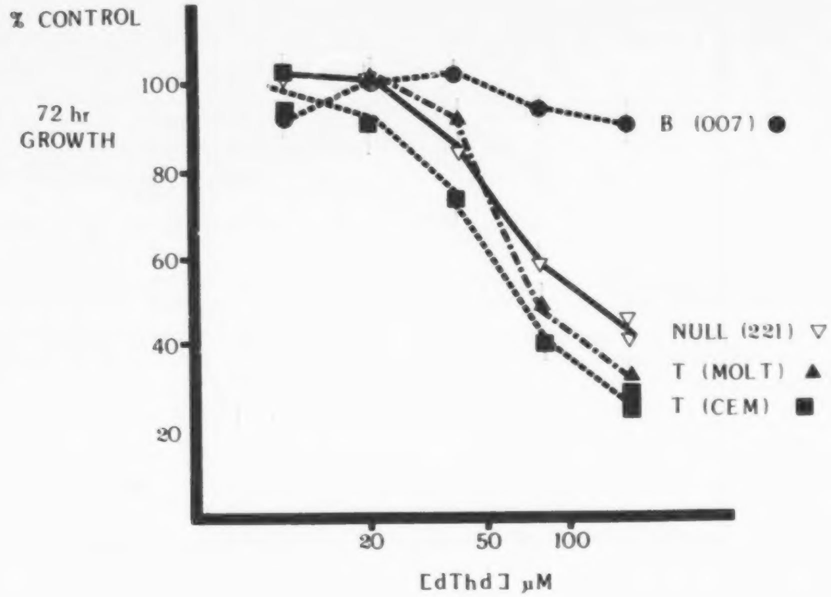

FIGURE 1.—Thymidine (dThd) dose-response toxicity curves in lymphoid cell lines. Cells in suspension were exposed in triplicate to varying thymidine concentrations for 72 hrs and then counted; growth is expressed as a % of control. Error bars = SE from at least 4 separate experiments. IC_{50} values (mean ± SE): LAZ 221 = 104 ± 10 μM; MOLT-4 = 82 ± 7 μM; CCRF-CEM = 51 ± 3 μM.

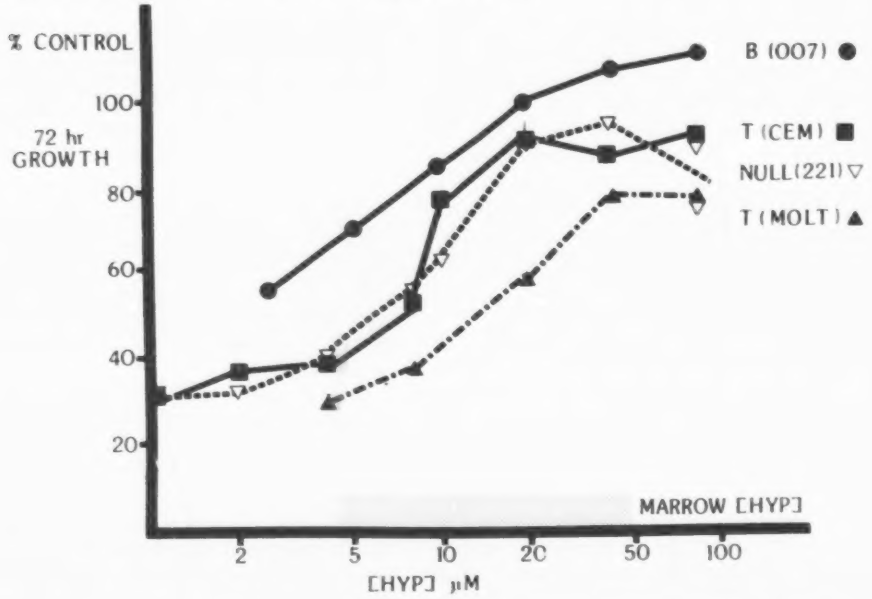

FIGURE 2.—Hypoxanthine (HYP) dose-response protection curves from methotrexate cytotoxicity. Cells were exposed in triplicate to varying hypoxanthine concentrations in the presence of 1.0 μM methotrexate and 20–40 μM thymidine for 72 hrs and then counted. Results are expressed as a % of control growth. Shaded area along abscissa corresponds to bone marrow hypoxanthine concentration range measured by Howell et al (16). Error bars = SE from at least 4 separate experiments. PC_{50} (mean ± SE): LAZ 007 = 6.2 ± 2.4 μM; LAZ 221 = 9.1 ± 1.4 μM; MOLT-4 = 15.2 ± 3.1 μM; CCRF-CEM = 8.5 ± 1.0 μM.

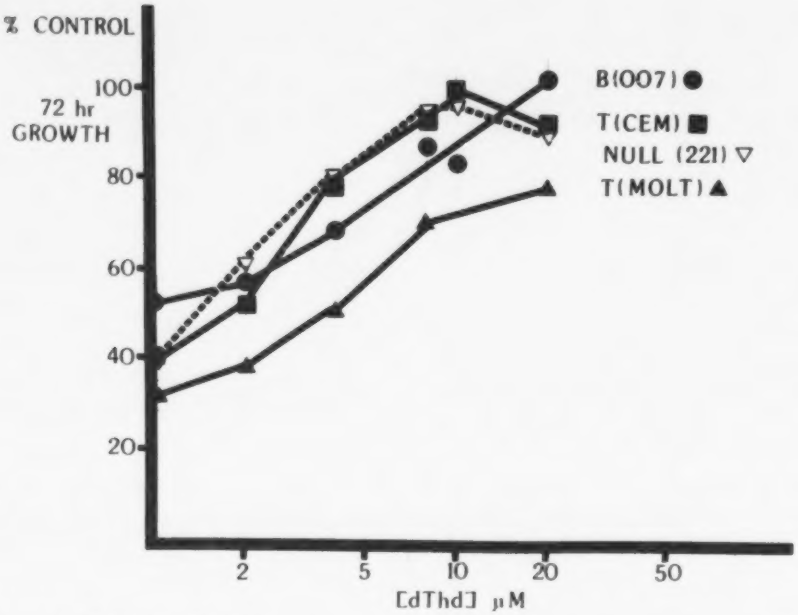

FIGURE 3.—Thymidine (dThd) dose-response protection curves from methotrexate cytotoxicity. Cells were exposed in triplicate to varying thymidine concentrations in the presence of 1.0 μM methotrexate and 80 μM hypoxanthine for 72 hrs and then counted. Results are expressed as a % of control growth. Error bars = SE from at least 4 separate experiments. PC_{50} (mean ± SE): LAZ 007 = 4.0 ± 0.9 μM; LAZ 221 = 2.2 ± 0.2 μM; MOLT-4 = 3.8 ± 0.5 μM; CCRF-CEM = 2.2 ± 0.3 μM.

The hypoxanthine requirements for methotrexate protection are quantitatively expressed as PC_{50} values in the legend. It is clear that hypoxanthine requirements vary among the cell lines. Of particular interest is the location of the steepest portion of all the dose-response curves, which falls within the physiologic concentration range for hypoxanthine as measured by Howell et al (16). This implies that small changes in hypoxanthine concentration may significantly influence methotrexate growth inhibition.

Figure 3 shows a dose-response relationship for thymidine protection in all cell lines when thymidine is added at the same time as methotrexate. The curves for CCRF-CEM (T cell) and LAZ 221 (null cell) are virtually superimposed. The absolute thymidine requirement is greatest for MOLT-4, as reflected by the rightward shift of the protection curve. However, the slope of the curve does not differ from that of the other cell lines. The thymidine dose-requirement curve for the B cell line LAZ 007 falls in an intermediate position. Although its slope is slightly flatter than those for the other cell lines, this difference is small. The relative thymidine requirements for methotrexate protection are expressed quantitatively as PC_{50} values in the legend. The results indicate that thymidine requirements for methotrexate protection measured in this way are not influenced by B, T, or null cell status.

Since the interaction between methotrexate and thymidine has been shown to be sequence-dependent (17), we extended these studies by prolonging the time interval between methotrexate and subsequent thymidine exposure in the B cell line (LAZ 007) and one T cell line (CCRF-CEM). We hypothesized that the delay in thymidine exposure would allow a progressive fall in the intracellular dTTP pool, thus unmasking any differences in thymidine utilization that were not apparent when thymidine and methotrexate were added simultaneously to cultures. A significant fall in dTTP levels would be expected within 4 hours of methotrexate exposure (14).

The thymidine dose-protection curves in these delay experiments were superimposed at all time points. Thus, a time-related shift in thymidine requirements for protection was not observed for either cell line up to 12 hours.

To further define measurable differences in the efficiency of thymidine protection, we calculated the mean maximum 72-hour growth achieved following each delay period and the mean thymidine concentration required to provide maximum growth (table 1). Threefold to fourfold more thymidine was required for maximum protection of the B cells versus the T cells at time points prior to 12 hours. At 12 hours, the difference in thymidine requirements between the cell lines was smaller but still significant. At the minimum thymidine concentration that maximally protected the B cells (40 µM), substantial toxicity was already observed in the CCRF-CEM cells, as shown in figure 5.

We compared maximum protection offered by thymidine and a single concentration of folinic acid by observing their abilities to support maximum growth in B (LAZ 007) and T (CCRF-CEM) cells. A single folinic acid concentration was studied since the intent was to use this form of rescue to control for differences not related to thymidine metabolism. The folinic acid concentration chosen (16 µM) was approximately 100 times the molar equivalent of methotrexate (0.2 µM). We had previously found, using another cell line, that almost complete protection could be achieved over a 72-hour growth period at this folinic acid:methotrexate concentration ratio (18).

The results comparing maximum thymidine and folinic acid protection (table 1) are graphically illustrated in figure 4. Folinic acid and thymidine (plus hypoxanthine) protection were almost complete (~ 100% of control growth) at zero time, when the rescue agents and methotrexate were added simultaneously to the cultures. As the folinic acid delay interval was progressively prolonged, there was a linear decline in the extent of protection in both cell lines. Folinic acid protection was superior in the B versus the T cell line at all time points.

As the thymidine delay interval was progressively prolonged, no decline in protection occurred until 12 hours in the B cell only. At the later time points thymidine (plus hypoxanthine) offered better protection than folinic acid in both cell lines.

The influence of methotrexate on thymidine-induced growth suppression was studied in the presence of hypoxanthine and escalating concentration of thymidine past the point of maximum protection. The methotrexate effect was similar for all cells and only that for CCRF-CEM is shown graphically (fig 5). Methotrexate added simultaneously with thymidine shifted the toxicity curve to the left, suggesting sensitization of the cells to thymidine. This sensitization is quantitatively expressed by comparing IC_{50} values for thymidine with and without methotrexate (*see* fig 5 legend). It is unclear why thymidine-induced growth suppression should be enhanced by simultaneous exposure to methotrexate. Simultaneous thymidine exposure may prevent a significant methotrexate-induced fall of dTTP levels by bypassing the de novo block in thymidylate synthesis. However, the rise in dTTP levels would not be expected to exceed the rise produced when exogenous thymidine is added without methotrexate.

In experiments in which thymidine exposure is delayed, a fall in intracellular dTTP levels after methotrexate treatment would be expected to shift the thymidine toxicity curve to the right since more thymidine would be required to attain an equivalent dTTP level. However, even under these circumstances, there is no evidence of an increased thymidine dose requirement to produce equivalent toxicity. Further discussion of these observations in light of the proposed mechanisms of methotrexate- and thymidine-induced growth suppression is presented below.

To evaluate the relative contribution of dCTP depletion to thymidine-induced toxicity in the presence and absence of methotrexate, we compared deoxycytidine

TABLE 1.—Comparison of thymidine and folinic acid protection in a B cell line (LAZ 007) and a T cell line (CCRF-CEM)*

		Maximum protection: % of control growth		
Time (hrs)†	Cell type	Thymidine	Folinic acid	Thymidine concentration (µM) for maximum protection
0	B	97 ± 2	102 ± 2	33 ± 10
	T	92 ± 2	90 ± 3	9.4 ± 0.3
1	B	98 ± 3	101 ± 3	32 ± 4
	T	91 ± 2	92 ± 1	9.1 ± 1.0
4	B	102 ± 7	95 ± 5	40 ± 16
	T	97 ± 2	83 ± 3	10 ± 0
6	B	103 ± 3	—	40 ± 8
	T	100 ± 2	83 ± 3	9.6 ± 1.0
12	B	89 ± 8	84 ± 3	26 ± 7
	T	104 ± 3	67 ± 5	12 ± 2

*Values = mean ± SE.

†From addition of methotrexate to thymidine or folinic acid exposure.

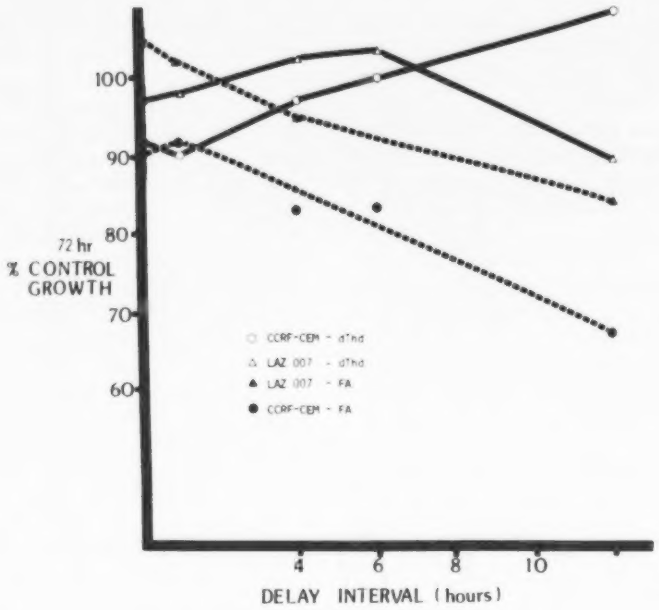

FIGURE 4.—Comparison of maximum protection from methotrexate toxicity offered by thymidine (dThd) and folinic acid (FA) in CCRF-CEM and LAZ 007. Cells were exposed in triplicate to 0.2 μM methotrexate, and for thymidine protection, 80 μM hypoxanthine was included. At 0, 4, 6, or 12 hrs after methotrexate exposure, 16 μM folinic acid or optimal concentrations of thymidine were added, and 72 hrs later cells were counted. Results are expressed as a % of control growth. Values for thymidine protection were calculated from the thymidine delay experiments described in the text. Error bars = SE from at least 4 separate experiments.

reversal under these conditions. The results (table 2) indicate that at 80 μM thymidine, a given concentration of deoxycytidine will provide excellent protection from toxicity in the presence and absence of methotrexate, although reversal is superior with methotrexate present. Studies in the murine system indicate that circulating deoxycytidine may be an important factor determining thymidine effects in vivo (19).

DISCUSSION

The studies described here were initiated to explore an in vitro model in which known differences in thymidine metabolism would be expected to differentially influence methotrexate toxicity during thymidine rescue. To control for factors that might contribute to differences in protection that were independent of thymidine metabolism, we compared the efficiency of thymidine and folinic acid rescue. We also studied the efficiency of hypoxanthine rescue and the influence of methotrexate on thymidine-induced growth suppression since these variables might also influence in vivo effects of thymidine and methotrexate treatment.

These studies failed to demonstrate significant differences in thymidine utilization for methotrexate protection among cells with widely different rates for thymidine catabolism except at concentrations needed for maximum protection. The thymidine concentration that maximally protects a B cell line (30–40 μM) is threefold to fourfold higher than that needed to protect a T cell line. However, this difference occurs at a thymidine level that provides substantial growth inhibition in all the T cell lines and is far above the in vivo level needed to protect against methotrexate toxicity (1,2,4). The net result of these effects is a substantial difference in growth between the cell lines, which is more closely related to thymidine suppression than to thymidine protection.

Substantial differences were observed between thymidine and folinic acid protection in the cell lines studied. While thymidine offered similar protection to both cell lines (except at 12 hours), folinic acid offered better protection to LAZ 007 than to CCRF-CEM at all time points. Secondly, the degree of protection offered by these agents in each individual cell line was quite different. In LAZ 007, thymidine and folinic acid produced

TABLE 2.—Deoxycytidine reversal of thymidine-induced growth inhibition in CCRF-CEM cells in presence and absence of methotrexate (+ hypoxanthine)

Drug	% of control growth, mean ± SE	
	Without deoxycytidine	With 80 μM deoxycytidine
Thymidine alone (80 μM)	25 ± 3	80 ± 1
Thymidine (80 μM) + methotrexate (0.2 μM)*		
0 hr†	22 ± 1	93 ± 4
1 hr	26 ± 2	93 ± 2
6 hr	25 ± 1	88 ± 3

*Growth in 0.2 μM methotrexate alone = –15% ± 1% of control growth.

†Delay interval between exposure to methotrexate (+ hypoxanthine) and addition of thymidine.

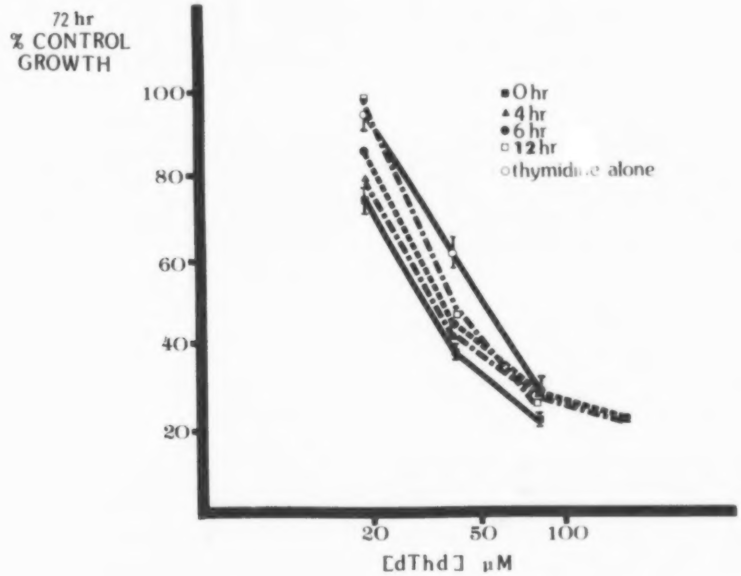

FIGURE 5.—Thymidine (dThd) dose-response cytotoxicity curves in the presence and absence of methotrexate. CCRF-CEM cells in triplicate were exposed to varying concentrations of thymidine alone or to 0.2 μM methotrexate. In methotrexate-treated cultures, 80 μM hypoxanthine was included, and varying concentrations of thymidine were added at 0, 4, 6, or 12 hrs after methotrexate exposure. Cells were counted 72 hrs after thymidine exposure and results are expressed as a % of control growth. Points represent mean values from at least 5 separate experiments. Error bars = SE. IC_{50} (mean ± SE): thymidine alone = 51 ± 3 μM; thymidine + methotrexate (0 hr) = 34 ± 3 μM.

equivalent protection, while in CCRF-CEM, thymidine protection was vastly superior to folinic acid protection. Such differences might be explained by cellular differences in susceptibility to an early methotrexate-induced purineless state (20), since hypoxanthine was included in thymidine-treated but not in folinic acid-treated cultures, or by reported differences in folate catabolism between malignant and nonmalignant cells (21). Further studies are in progress to characterize the differences in folinic acid protection seen with these cell lines.

In contrast to the observations on thymidine protection, differences in hypoxanthine protection among cells and the steep portions of the hypoxanthine protection curves did occur at physiologic hypoxanthine concentrations, indicating that small differences in hypoxanthine concentration might have profound effects on cell growth. Since blood and bone marrow hypoxanthine levels differ significantly among individuals (16), the selective antitumor effects of methotrexate during thymidine rescue may be closely related to purine levels and purine salvage capability. Studies in the murine system support a role for endogenous purines in modulating in vivo effects of methotrexate (22).

The clinical use of methotrexate and thymidine in combination might be considered for tumors sensitive to both agents. Therefore, we examined the effects of methotrexate (plus hypoxanthine) on thymidine-induced growth inhibition. Thymidine inhibition depends upon an expanded dTTP pool while methotrexate depletes this pool. We thus expected that in the presence of methotrexate, excess thymidine might be required to produce toxic effects equivalent to those produced by thymidine alone. This might be especially true in circumstances in which thymidine exposure is delayed since a progressive fall in intracellular dTTP pools would occur prior to addition of thymidine. However, the opposite phenomenon was observed (fig 5); the thymidine suppression curve was shifted to the left in the presence of methotrexate. The observations on deoxycytidine reversal of thymidine toxicity in the presence and absence of methotrexate may provide a clue to the explanation of thymidine sensitization by methotrexate. Deoxycytidine (which has no effect on methotrexate growth suppression in the absence of thymidine) substantially reversed thymidine toxicity both in the presence and absence of methotrexate (table 2). This suggested that the toxicity observed in the presence of methotrexate was in large part due to thymidine. However, methotrexate will also cause a decline of dCTP pools, which is thought to be responsible for thymidine toxicity. This methotrexate effect depends upon dTTP depletion with subsequent release of its natural inhibition on dCMP deaminase. This in turn allows more dCMP to be converted to dUMP rather than dCTP (23). Thus, a paradoxical situation exists in which dCTP depletion in the presence of methotrexate depends upon *low* dTTP levels, while in the presence of thymidine, *high* dTTP levels result in dCTP depletion. The superior reversal of thymidine toxicity by deoxycytidine in the presence of methotrexate is unexplained but could be related to changes in purine deoxynucleoside triphosphate pool sizes in the presence and absence of methotrexate. Modulation of purine ribonucleotide reductase by dTTP results in enhanced dGDP formation and elevated dGTP pools. Grindey et al (12,24) have argued that elevated dGTP pools may contribute to thymidine toxicity through modulation of a regulatory protein for DNA polymerase. Thus, thymidine may inhibit DNA synthesis, and subsequently cell growth, through depletion of dCTP as well as through elevation of dGTP. However, the addition of methotrexate, which has an antipurine effect, may prevent dGTP from rising to inhibitory levels, especially in the presence of a low concentration of salvageable purine. Therefore, in the presence of methotrexate, dCTP depletion would play a proportionally larger role in inhibiting cell growth, and deoxycytidine reversal would be more efficient. In a similar manner, the modulation of thymidine toxicity by methotrexate could be influenced by the concentration of salvageable purine present, since this would influence the size of the dGTP pool and its relative contribution to thymidine toxicity. Measurement of dNTP pool sizes will be required to substantiate such mechanisms.

The results of the in vitro studies reported here point out the complexities that accompany attempted modulation of drug effects.

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Activity of 4′-Epi-doxorubicin on Normal Hematopoietic Precursor Cells in Mice[1,2]

Alberto Sobrero,* Carlo Muzzulini, Francesco D'Amore, Giuseppe Bogliolo, Giulio Massa, Riccardo Ghio, and Ivo Pannacciulli[3,4]

The effects of 4′-epi-doxorubicin (4′-epi-DX) on survival of in situ murine bone marrow hematopoietic precursor cells (HPC) were studied. The drug was given to (C57BL × C3H)F$_1$ mice in increasing single doses, in repeated administrations (three times in 5 days), or for a long period (twice a week for 10 weeks). Survival of colony-forming units (CFU$_s$, spleen; CFU$_c$, culture; and CFU$_e$, erythroid) was assayed by in vivo and in vitro methods; dose survival curves were exponential. It appears that single doses of 4′-epi-DX have a greater impact on murine HPC than single doses of the parent compound. Following repeated administrations, pluripotential stem cells recover better after the derivative than after its parent drug. During prolonged treatment, the bone marrow content of committed progenitors is depleted to a lesser extent in the animals treated with 4′-epi-DX than in those receiving DX. It is, however, possible that, following the same prolonged treatment, this difference is minimal. In conclusion, these experimental data seem to indicate that 4′-epi-DX has lower toxicity in murine HPC than DX and that this is evident mainly after repeated administrations for a short period of time. [Cancer Treat Rep 66:2061–2070, 1982]

The cytotoxic effect of doxorubicin (DX) on normal hematopoietic cells, together with its serious cardiac toxicity, is a major problem in the clinical use of this drug. Various anthracycline derivatives (1–6) are being studied in an effort to find a compound with an increased therapeutic index. 4′-Epi-doxorubicin (4′-epi-DX) appears to be of particular interest, since both in experimental and clinical studies (7–13) it has shown antitumor activity at least equal to that of the parent compound and has had somewhat lower myocardial and hematologic toxicity. Recently, Lohrmann and Schreml (14) reported several studies on the effects of anthracyclines on mouse hematopoietic precursor cells (HPC). Marsh (15) has shown that the sensitivity of bone marrow hematopoietic progenitors to DX is different from their sensitivity to *N*-trifluoro-acetyladriamycin-14-valerate, another analog of DX, and that this difference may vary according to the animal species studied. In a recent study (16) we compared the effects of DX to those of 4-demethoxydaunorubicin on murine hematopoietic stem cells. In light of this and previous research (17,18) it seems that the assay of hematopoietic stem cells in animals treated with new anthracycline analogs is a useful means for assessing their potential myelotoxicity. The purpose of the study reported here was to evaluate the effects of 4′-epi-DX on survival of in situ bone marrow stem cells following the injection of increasing single doses of the drug or during its repeated or prolonged administration.

MATERIALS AND METHODS

Mice

All experiments were performed on (C57BL♂ × C3H♀)F$_1$ adult mice, 8–12 weeks old. Recipient mice were irradiated with a Theratron Junior cobalt-60 machine (80 rads/minute; total dose, 900 rads).

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[1] Received Mar 10, 1981; revised July 9, 1982; accepted Sept 2, 1982.

[2] Supported in part by CNR contracts 15/79.00653.96/115.4751 and 101/78.02866.96/115.4823.

[3] Cattedra di Patologia Speciale Medica RR (*A. Sobrero, C. Muzzulini, F. D'Amore, G. Bogliolo, G. Massa*, and *I. Pannacciulli*) and Cattedra di Terapia Medica (*R. Ghio*), Istituto Scientifico di Medicina Interna, University of Genoa, Italy.

[4] We thank Prof. A. M. Casazza for helpful suggestions and criticism, Mr. Kenneth Dinnen for preparation of the manuscript, and Ms. B. Johnston for editing the manuscript.

**Reprint requests to:* Dr. Alberto Sobrero, Istituto Scientifico di Medicina Interna, University of Genoa, Viale Benedetto XV 6, 16132 Genoa, Italy.

Drugs

DX and 4′-epi-DX (IMI 28, batch 10024030) hydrochlorides were supplied by Farmitalia-Carlo Erba (Milan, Italy) in the form of red powder. They were stored in the dark in a desiccator at 4°C and dissolved in normal saline (NaCl, 0.9%) at the working concentration immediately before use. Structure, molecular weight, and biologic and pharmacologic activities of 4′-epi-DX have already been reported (7,8,19,20). Both DX and 4′-epi-DX were injected into the tail veins of mice. Only in the case of prolonged administration (10 weeks) were the drugs given ip.

Experimental Project

To fully evaluate the hematologic toxicity of 4′-epi-DX a comparison was made with the parent drug DX. According to previous experimental evidence (7–9), DX and 4′-epi-DX have the same antitumor effect at the same dose; 4′-epi-DX, however, has a much higher therapeutic index than DX. Throughout this study, which was aimed at assessment of hematologic toxicity, comparison between the two anthracyclines was made at equipotential but not equitoxic doses, that is, at equal doses of each drug.

Experiment A: in vivo sensitivity of murine HPC to 4′-epi-DX.—The aim of this experiment was to evaluate the shape and slope of in vivo dose-survival curves of colony-forming units (CFU_s, spleen; CFU_c, culture; and CFU_e, erythroid) in mice treated with 4′-epi-DX. Statistical evaluation of the results allows a comparison between the different levels of toxicity of 4′-epi-DX on the different hematopoietic populations. Groups of five mice each received single iv injections of 4′-epi-DX at various dose levels: that is, 1.6, 2.7, 4.5, 6.8, 11.5, 19.0, and 31.6 mg/kg of body weight. These doses (each $^5/_3$ of the previous one) were selected, taking into account that the LD50 in healthy CDF_1 mice at 1 month for 4′-epi-DX is 18.52 mg/kg (7). Twenty-four hours after injection, the mice were bled from the orbital sinus for determination of packed rbcs, the total wbc count per liter, and the number of reticulocytes per 100 rbcs; then they were killed by cervical dislocation. Both femurs were removed, bone marrow (bm) cellularity was estimated, and bm cell suspensions for HPC assays were prepared. Results were compared with those obtained in matched groups of mice that received equal doses of DX and that were treated and assayed in exactly the same way. These results have already been partially reported (16). Normal, untreated controls injected only with saline were also tested.

Experiment B: time-survival curves for bone marrow HPC in mice treated with repeated doses of 4′-epi-DX.—According to Casazza (1) a schedule of intermittent treatment with 4.5 mg/kg of DX at time 0 and on Days 2 and 5 was particularly useful for enhancing the antileukemic activity of DX in Gross' leukemia-bearing C3H mice. This schedule was applied to define all possible differences in hematologic toxicity between 4′-epi-DX and DX when the two drugs are administered in repeated doses. Accordingly, two groups of 50 mice received 4.5 mg/kg iv of either DX or 4′-epi-DX at time 0 and on Days 2 and 5. On Days 2, 3, 5, 6, 8, 9, 12, 15, 18, and 21, five treated mice from each group and five saline-treated controls were killed shortly after the determination of the above-mentioned peripheral blood cell counts. The assays for progenitor cells were performed on bm cell suspensions.

Experiment C: time-survival curves for bone marrow HPC in mice during long-term administration of 4′-epi-DX.—Long-term administration of anthracyclines leads to a severe cardiotoxic effect. The minimal cumulative cardiotoxicity of 4′-epi-DX, however, is higher than that of DX (8). The aim of this experiment was to see whether equal cumulative doses of 4′-epi-DX and DX have different hematologic toxic effects. Accordingly, two groups of 80 mice were treated twice a week ip with 2.5 mg/kg either of DX of 4′-epi-DX, given for 10 weeks. At the end of each week, the determinations on peripheral blood and bm described above were performed in five treated mice from each group and in five saline-treated controls.

Methods

The peripheral blood cell counts were carried out by conventional methods (21). Bm collection and preparation of single-cell suspensions for CFU assays were performed by the usual techniques (22).

Assays for hematopoietic CFU.—A first aliquot of the cell suspension was assayed for CFU_s content (23). Nine mice, irradiated as reported above, were used as recipients. A total of 0.5 ml of cell suspension, diluted in medium to contain 4×10^4 bm cells, was injected into tail vessels. The host mice were killed with ether 9 days after the injection, and their spleens were excised and fixed in Tellyesniczky's fluid. The number of visible colonies was then counted. The mean number of colonies in each spleen was calculated, and the total number of CFU_s per femur ± SE was determined. A second aliquot of the cell suspension was assayed for CFU_c content (22). One ml of agar α-medium containing 1×10^5 bm cells and 20% horse serum was pipetted into triplicate 35-mm Falcon Petri dishes over 0.1 ml of colony-stimulating factor prepared from an extract of the uterine membrane of a pregnant mouse. The cultures were incubated at 37°C in a 95%–100% humidified atmosphere of 5% CO_2 in air. Cultures were examined after 7 days of incubation, using an inverted microscope. The number of colonies containing > 50 cells was

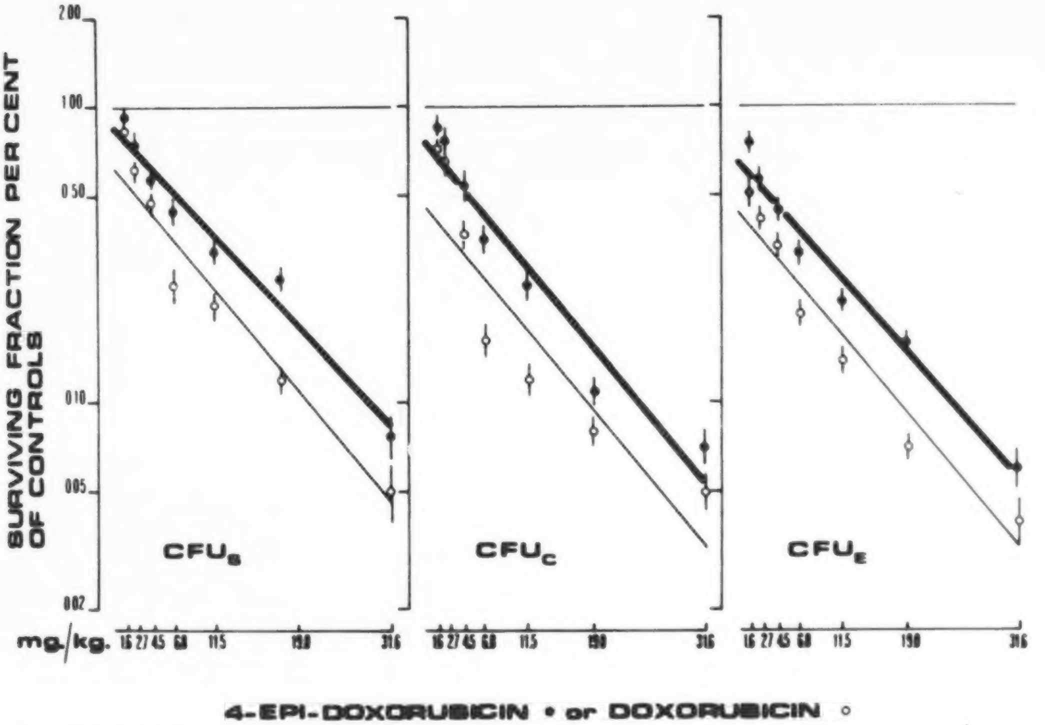

FIGURE 1.—Dose-survival curves of bm HPC 24 hrs after 4′-epi-DX or DX single injection. For each experimental point, vertical bars = mean ± SE (normal values/femur in untreated mice: CFU$_s$ = 3934 ± 92; CFU$_c$ = 14,162 ± 635; CFU$_e$ = 14,560 ± 654). 4′-Epi-DX: CFU$_s$, r = −0.984 ($P < 0.01$); CFU$_c$, r = −0.968 ($P < 0.01$); CFU$_e$, r = −0.979 ($P < 0.01$). DX: CFU$_s$, r = −0.967 ($P < 0.01$); CFU$_c$, r = −0.968 ($P < 0.01$); CFU$_e$, r = −0.973 ($P < 0.01$).

recorded, and the mean number of CFU$_c$ per femur ± SE was calculated. A third aliquot of the same cell suspension was assayed for its CFU$_e$ content (24). A total of 1 × 10^5 nucleated bm cells in 1 ml of a mixture containing 0.8% methylcellulose, 30% horse serum, and 0.25 units of erythropoietin were plated into triplicate 35-mm Falcon Petri dishes. The erythropoietin was prepared using plasma from phenylhydrazine-treated sheep. The cultures were incubated for 3 days in the conditions described above. Only those colonies containing more than eight cells were scored, and the mean number of CFU$_e$ per femur ± SE was then determined.

Evaluation of results.—In treated mice and controls, single data points were examined on the same day by the same assay, to minimize possible day-to-day variations of the assays. In time-response experiments, the differences between data obtained in 4′-epi-DX- or DX-treated mice and between each of these groups and controls were evaluated by Student's t test. In the figures, the values of CFU$_s$, CFU$_c$, and CFU$_e$ per femur were normalized to the untreated controls. The absolute numbers of HPC, bm cells, wbcs, and reticulocytes in controls are included in the legend of figure 1 and in table 1. The linear regressions between drug doses and survival fractions were fitted by the least squares method. Linear regression coefficients (r) and their significance were calculated. Comparison among sensitivities of each population assayed to each drug was performed by analysis of variance (F variance ratio and its significance) (25).

RESULTS

General Toxicity

In experiment B, the spontaneous death rate during the 10-day period of observation after treatment with three doses of 4.5 mg/kg was 3% in the DX-treated mice and 0 in the 4′-epi-DX-treated animals. No severe toxic signs were observed in surviving mice. During the 10 weeks of experiment C, spontaneous deaths were observed in 10% of the DX-treated mice and in 3% of the 4′-epi-DX group. Toxic signs were not evident in the survivors.

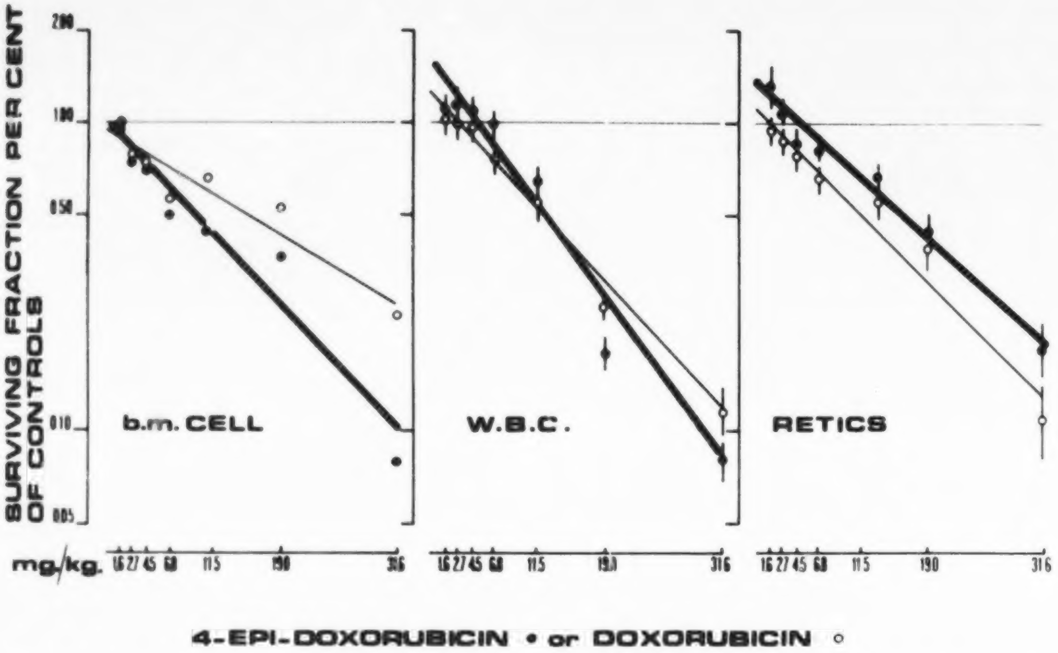

FIGURE 2.—Dose-survival curves of bm and peripheral blood cells 24 hrs after 4′-epi-DX and DX single injection. For each experimental point, vertical bars = mean ± SE. 4′-Epi-DX: bm cells, r = –0.967 ($P < 0.01$); wbc counts, r = 0.980 ($P < 0.01$); reticulocyte counts (retics), r = –0.986 ($P < 0.01$). DX: bm cells, r = –0.949 ($P < 0.01$); wbc counts, r = –0.994 ($P < 0.01$); reticulocyte counts, r = –0.986 ($P < 0.01$).

Dose-Survival Curves of Peripheral Blood Cells, Bone Marrow Cells, and HPC 24 Hours After 4′-Epi-DX Single Injection

Results are reported in figures 1 and 2. After 4′-epi-DX, within the dose range used, the survival curves for every cell population assayed were exponential (for each regression coefficient, $P < 0.01$). After comparison by analysis of variance it was seen that the slopes of the survival curves for CFU_s, CFU_c, CFU_e, wbcs, and reticulocytes showed a similar sensitivity ($P > 0.05$). On the other hand the dose-survival curve of bm cells indicated a higher sensitivity ($P < 0.01$). However, values obtained by calculation of the level at which the exponential line crosses the abscissa were 87%, 78%, and 66% of survival for CFU_s, CFU_c, and CFU_e, respectively, and 99%, 154%, and 129% for bm cells, wbcs, and reticulocytes, respectively. The lower extrapolation numbers for HPC may indicate that at low doses 4′-epi-DX has a greater impact on stem cells than on more mature cells. A comparison by analysis of variance of the dose-survival curves after 4′-epi-DX and after DX single doses showed similar slopes in the two groups ($P < 0.05$). However, with regard to HPC, the extrapolation numbers for DX (62%, 45%, and 44% of survival for CFU_s, CFU_c, and CFU_e, respectively) were lower than for 4′-epi-DX, while differences were much less marked for bm cells, wbcs, and reticulocytes (94%, 128%, and 111%, respectively) in DX-treated mice.

Moreover, considering single experimental points, there is a statistically significant difference (Student's t test: $P < 0.01$) between survival fractions of HPC of the two groups at the following doses: CFU_s—4.5, 6.8, and 19 mg/kg; CFU_c—4.5, 6.8, and 11.5 mg/kg; and CFU_e—2.7, 4.5, 6.8, 19.0, and 31.6 mg/kg. No statistically significant difference was detectable for the other parameters, except for wbcs at a dose of 6.8 mg/kg and reticulocytes at 1.6 mg/kg.

Effects on Peripheral Blood Cells, Bone Marrow Cells, and HPC of an Intermittent Treatment Schedule With 4′-Epi-DX

The survival of HPC, bm cells, wbcs, and reticulocytes as a function of time during the following intermittent administration of 4′-epi-DX is represented in figure 3 and reported in table 1 together with the sequence for mice treated with the same schedule and dose of DX. The pattern of CFU_s changes was characterized by an initial (Day 3) severe depletion followed by a prompt recovery (Day 6), overshooting the normal level. The administration of the third dose of the drug

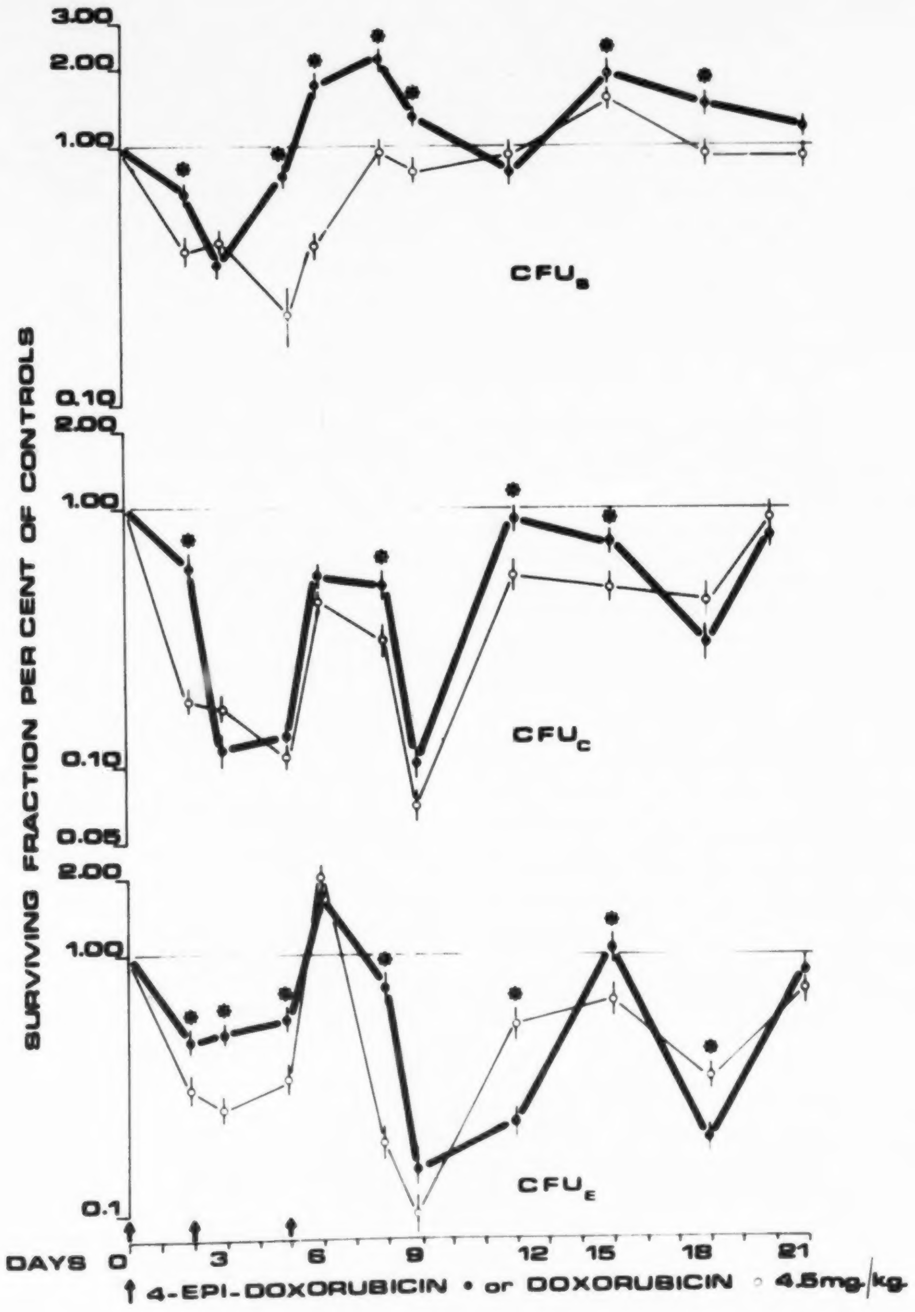

FIGURE 3.—Fractional survival of CFU as function of time during and after intermittent treatment schedule with DX and 4′-epi-DX. For each experimental point, vertical bars = mean ± SE. Values marked with asterisk are significantly different ($P < 0.05$) from corresponding values (○) of DX-treated animals.

TABLE 1.—Effects of an intermittent

	Values in untreated mice	Drug (4.5) mg/kg	2	3	5
VPRC (%)	44 ± 0.8	4′-Epi-DX	44 ± 0.9	43 ± 1.0	45 ± 0.3
		DX	41 ± 2.4	44 ± 1.2	45 ± 1.7
Wbc count (× 10^9 cells/L)	5.0 ± 0.2	4′-Epi-DX	4.9 ± 0.3	3.8 ± 0.4	2.0 ± 0.1†,‡
		DX	5.5 ± 0.3	4.3 ± 0.7	4.0 ± 0.1†
Reticulocyte count (cells/100 rbcs)	2.7 ± 0.3	4′-Epi-DX	2.3 ± 0.2	2.4 ± 0.2	3.3 ± 0.1
		DX	2.2 ± 0.2	2.3 ± 0.2	3.0 ± 0.1
Bm cellularity (cells × 10^6)	13.5	4′-Epi-DX	7.8	12.0	14.4
		DX	8.0	7.3	9.2

*Values = mean ± SE. VPRC = volume of packed rbcs.
†Values are significantly different ($P < 0.05$) from those found in untreated mice.
‡Values are significantly different ($P < 0.05$) from those found in DX-treated mice.

did not interfere with this recovery. The pattern of CFU_c and CFU_e changes in the first 6 days of observation was similar to that of CFU_s changes, but the initial recovery was transitory, and a second much more severe reduction of CFU_c and CFU_e took place. This was followed by a second recovery, a third drop, and finally, a return to normal values. The overall pattern was one of oscillations at and below the normal level. In DX-treated mice the drop of CFU_s was more rapid and their recovery slower. There was no initial phase of overshooting. The sequence of CFU_c and CFU_e changes was, however, similar to that observed in 4′-epi-DX-treated animals with minor, although statistically significant differences; this is evidence that DX also has a more marked effect on committed progenitors. In bm cellularity and peripheral blood cell counts (table 1), the sequences of changes during and following 4′-epi-DX or DX are similar with few, inconsistent statistically significant differences. At the end of the experimental period (Day 21), every blood parameter checked was normal again.

A schedule of intermittent treatment with 4′-epi-DX appeared to have less effect on HPC, and pluripotential stem cells in particular, than a similar schedule of DX. The effects of the two schedules on bm cellularity and on peripheral blood cell counts, however, were almost the same.

Effects on Peripheral Blood Cells, Bone Marrow Cells, and HPC of Prolonged Treatment With 4′-Epi-DX Twice a Week

As shown in table 2, the sequence of the changes of peripheral blood cell counts and of bm cellularity observed during prolonged 4′-epi-DX administration showed only minor or transitory differences, compared with those induced by DX. In both DX- and 4′-epi-DX-treated animals, the main feature was an almost constant reduction of the wbc counts, which reached a severe degree at Weeks 4, 9, and 10. This was accompanied by a slight but constant decrease of the volume of packed rbcs. The CFU_s content of the bm (fig 4) remained normal or reached values significantly above normal in both groups of mice, except for a mild reduction at Week 10 in 4′-epi-DX-treated mice and at Weeks 1 and 10 in DX-treated animals. In DX-treated mice the CFU_c and CFU_e

TABLE 2.—Effects of prolonged 4′-epi-DX

	Values in untreated mice	Drug (2.5 mg/kg)	1	2	3
VPRC (%)	44 ± 0.8	4′-Epi-DX	42 ± 0.6	39 ± 0.6†	34 ± 0.6†,‡
		DX	38 ± 0.9†	38 ± 0.8†	39 ± 0.4†
Wbc count (× 10^9 cells/L)	5.0 ± 0.2	4′-Epi-DX	3.1 ± 0.3†	4.2 ± 0.9	3.4 ± 0.3†
		DX	3.0 ± 0.2†	4.8 ± 0.6	3.5 ± 0.1†
Reticulocyte count (cells/100 rbcs)	2.7 ± 0.3	4′-Epi-DX	3.5 ± 0.4	3.9 ± 0.2†,‡	3.1 ± 0.3‡
		DX	3.2 ± 0.4	2.4 ± 0.3†	3.4 ± 0.2
Bm cellularity (cells × 10^6)	13.5	4′-Epi-DX	14.0	14.0	13.2
		DX	8.0	11.0	14.0

*Values = mean ± SE. VPRC = volume of packed rbcs.
†Values are significantly different ($P < 0.05$) from those found in untreated mice.
‡Values are significantly different ($P < 0.05$) from those found in DX-treated mice.

4′-epi-DX and DX schedule*

Treatment Day						
6	8	9	12	15	18	21
41 ± 0.6	40 ± 0.9	41 ± 1.7	29 ± 1.9†	34 ± 3.0	38 ± 1.2	46 ± 1.6
42 ± 0.6	40 ± 0.6	41 ± 1.2	33 ± 2.3†	39 ± 2.7	40 ± 0.9	40 ± 2.1
4.3 ± 0.1	7.5 ± 0.3†,‡	3.7 ± 0.6	5.3 ± 0.3	2.4 ± 0.2†	4.3 ± 0.4	6.3 ± 0.4
5.4 ± 0.8	3.4 ± 0.2†	3.9 ± 0.2†	3.4 ± 0.4†	3.8 ± 0.2	2.8 ± 0.3†	5.3 ± 0.4
4.2 ± 0.3†	3.3 ± 0.1	2.7 ± 0.2	4.3 ± 0.2†,‡	6.0 ± 0.4†,‡	2.5 ± 0.2‡	3.0 ± 0.3
3.4 ± 0.2	3.2 ± 0.1	3.1 ± 0.2	2.0 ± 0.1	4.1 ± 0.3†	1.4 ± 0.2†	2.6 ± 0.2
12.6	23.0	9.0	7.2	19.2	8.6	16.7
11.2	10.0	6.6	10.1	23.1	7.8	20.1

content of the bm was just below or well below the normal value, for most of the observation period. Steep falls alternated with abortive or transitory recoveries. At Week 10 of treatment the bm content of CFU$_c$ was at its lowest level. In 4′-epi-DX-treated mice, the general trends of CFU$_c$ and CFU$_e$ were similar, but their levels were generally higher than in the DX group. Five of ten CFU$_c$ determinations were in or above the normal range. However, at Week 10 of treatment, in both groups of animals, there was a severe depletion of committed progenitors, which contrasted with the nearly normal endowment of the pluripotential stem cells.

DISCUSSION

The antitumor activity of 4′-epi-DX has been evaluated on experimental tumors and more recently also in clinical trials. In mice with leukemias L1210 or P388, Gross' leukemia, solid Sarcoma 180, or transplanted early and advanced mammary carcinoma (7–9), the antitumor effect of 4′-epi-DX was comparable to that of DX. The analog, however, was better tolerated. Evaluating new anthracycline compounds by the human tumor stem cell assay, Salmon et al (10) have shown that 4′-epi-DX and DX have almost the same potency of inhibition (46%–47%). In humans, too, the anticancer values of 4′-epi-DX and DX were substantially comparable, the new analog having the advantage of a lower incidence of marrow suppression and of other side effects (11). In another study (12), 4′-epi-DX showed a less marked hematologic toxicity and a more rapid elimination than DX. Preliminary results of a phase II trial of 4′-epi-DX for advanced solid tumors (13) indicated that overall efficacy of the drug is similar to that of DX in breast cancer. Again, the new analog appeared to be less toxic than the parent drug. These results tend to suggest that 4′-epi-DX is a valid alternative to DX. This is further supported by the data showing that, in mice, 4′-epi-DX seems to have a lower impact on HPC than DX. When the toxic effect is assessed 24 hours after a single dose of either drug, the slopes of the survival curves for the three types of HPC assayed in 4′-epi-DX-treated mice are statistically similar to those for DX-treated mice. The lower extrapolation levels at which the exponential lines cross the abscissa in 4′-epi-DX-treated animals and the statistically significant differences be-

and DX treatment twice a week*

Treatment Week						
4	5	6	7	8	9	10
34 ± 0.6†,‡	31 ± 1.4†,‡	37 ± 0.6†	39 ± 0.6†	35 ± 0.3†	34 ± 0.6†	30 ± 0.3†
39 ± 0.6	38 ± 0.4†	37 ± 0.6†	37 ± 0.6†	33 ± 2.0†	34 ± 1.4†	25 ± 0.3†
2.6 ± 0.3†	3.4 ± 0.5†	3.5 ± 0.2†	3.4 ± 0.5†	3.6 ± 0.4†	2.8 ± 0.4†	2.6 ± 0.3
2.4 ± 0.3†	3.9 ± 0.6†	4.5 ± 0.2	3.2 ± 0.3†	3.4 ± 0.3†	2.5 ± 0.2†	3.0 ± 0.6
3.6 ± 0.4†	4.4 ± 0.6†	3.4 ± 0.3	3.2 ± 0.2	2.5 ± 0.4‡	2.5 ± 0.4	1.3 ± 0.1†,‡
3.1 ± 0.6	4.2 ± 0.2†	3.7 ± 0.3†	3.3 ± 0.2	3.3 ± 0.2	2.4 ± 0.3	2.0 ± 0.1†
9.4	12.8	15.4	12.8	9.6	15.1	5.0
10.0	10.0	11.6	10.4	6.7	14.3	5.1

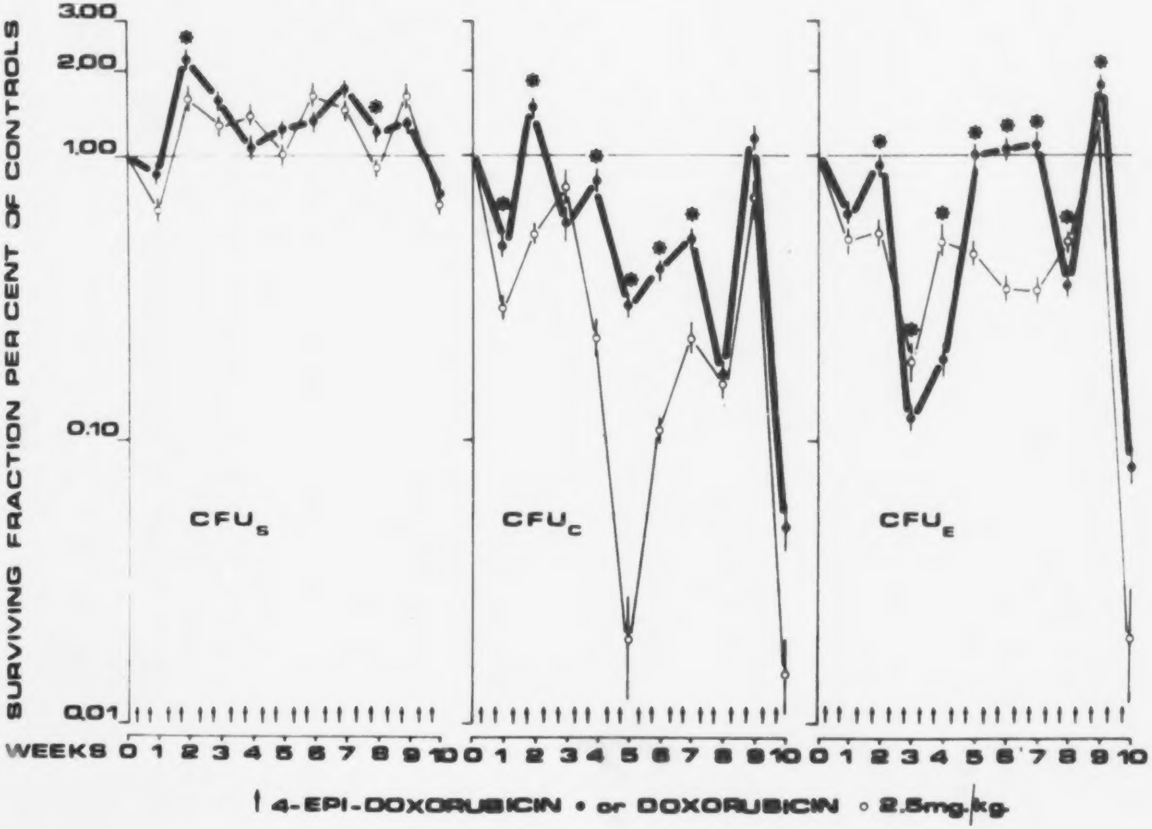

FIGURE 4.—Fractional survival of CFU as function of time during treatment with DX and 4'-epi-DX twice/wk. For each experimental point, vertical bars = mean ± SE. Values marked with asterisk are significantly different ($P < 0.05$) from corresponding values (○) of DX-treated animals.

tween survival fractions at most single experimental points, however, indicate that the effect of 4′-epi-DX on HPC is less toxic. On the contrary, there are no or only minor differences in bm cellularity and wbc and reticulocyte counts. Also, when the drugs were administered on an intermittent schedule, the pluripotential stem cells and, to a lesser degree, the committed stem cells seemed to be less affected by 4′-epi-DX than by DX. Finally, when the drugs were given for a relatively long period, the difference between 4′-epi-DX and its parent compound was particularly evident as far as the committed stem cells were concerned, while the milder involvement of the CFU_s prevented the detection of any possible differences of sensitivity to the two drugs. Any discussion of the difference between the effects of DX and its derivative 4′-epi-DX can only be a matter of conjecture. The survival-dose curves of precursor cells for both drugs are exponential, indicating that both DX and 4′-epi-DX are cycle phase nonspecific agents. Moreover, CFU_s are primarily a resting population, whereas committed progenitors (CFU_c and CFU_e) include a much higher proliferating fraction. Differences in the effects of DX and 4′-epi-DX were observed, however, for all three types of cells. These considerations seem to rule out the possibility that the kinetics of the target cells are conclusive in accounting for these differences.

4′-Epi-DX has a distribution in the tissues similar to that of DX (7), but it is retained less in the organs. If one assumes that recruitment of resting cells begins in the 24 hours following drug administration, the cell entering in proliferation could be differently affected if exposed to a different tissue level of the cytotoxic drug. Thus, the prompter reduction of tissue level of 4′-epi-DX compared with DX could lead to a lowering of the toxicity on recruited cells and account for what seems to be a less marked effect of 4′-epi-DX on HPC.

It is also possible that more subtle biologic differences between the two drugs are responsible for the differences in toxic effects on the very immature hematopoietic stem cells. Di Marco et al (26) showed that differences in polarity and in pKa values between DX and 4′-epi-DX are related to their different antitumor activity and toxicity in leukemia P388-bearing mice and to their uptake by leukemia L1210 cells. The finding that after 4′-epi-DX the dose-survival curve for bm cellularity shows a greater decline than after DX is very intriguing, particularly if one considers the concomitant absence of consistent differences in peripheral blood parameters. No ready explanation seems available at the moment, and more experiments will be necessary to clarify this result.

When cytotoxic drugs are administered for a relatively long time, a very intricate interplay of opposing factors must be taken into account. Among these, the cumulative toxicity of repeated doses of drugs, on the one hand, and the compensating mechanisms operating inside the target populations, on the other hand, are of primary importance. This complexity of changes taking place both inside and outside the hematopoietic system does not allow a valid analysis of the sequence of progenitor cell variations observed during and following intermittent and prolonged administration of the anthracyclines studied. However, the general trend of results obtained open the door to some significant considerations.

In the short schedule of intermittent treatment (experiment B), 4′-epi-DX shows a lower toxic effect than DX on CFU_s and also partially on CFU_c and CFU_e, without consistent differences in blood parameters. This might suggest a similar effect of both drugs on more advanced stages of myelopoiesis and, consequently, a similar immediate hematotoxic pattern.

In 4′-epi-DX-treated mice, however, the self-renewing capacity of the hematopoietic system is partially spared, and these animals have a greater chance of full recovery from the drug insult. Thus, the long-term results obtained with this short schedule of treatment, which assures a lower general toxicity and greater antileukemic activity of DX, both in normal and tumor-bearing animals (1), could be further improved by substituting 4′-epi-DX for DX.

In the experiment of long-term toxicity (10 weeks), both drugs caused a similar reduction of wbc count, but 4′-epi-DX had a less marked effect on committed stem cells than DX. This again is evidence of similar action of the two drugs in the more advanced stages of myelopoiesis and of a lesser toxicity of 4′-epi-DX in the earlier phases of hematopoiesis. Thus, prolonged administration of 4′-epi-DX, while causing an apparent hematologic toxicity of the same degree as DX, may in fact have a lesser effect on the progenitor compartments, thus allowing longer treatment. Results obtained after 10 weeks of treatment may, however, indicate that, in the long run, the two drugs reach an equal degree of toxicity on stem cells. This may suggest that the lesser toxicity of 4′-epi-DX is exploitable only with a short schedule of treatment.

Previous experience (27–29) indicates that there are discrepancies between the effects of cytotoxic drugs on morphologically recognizable hematopoietic cells and on colony-forming precursors. In this study, most of the results show that there are no great differences between the effects of DX and 4′-epi-DX on peripheral blood cell counts or bm cellularity. However, the difference becomes evident at the HPC level. It appears, therefore, that HPC assays, when possible, would constitute a much more sensitive means for assessing the degree of drug myelotoxicity in man.

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Cytotoxic and Cell Kinetic Effects of 3,4,5-Trihydroxybenzohydroxamic Acid (VF 122) in Hepatoma 3924A Cells[1,2]

Jasna Ban,[3,4] **Edith Olah,**[3,5] **and George Weber**[3,*]

3,4,5-Trihydroxybenzohydroxamic acid (VF 122), an inhibitor of ribonucleotide reductase, killed rat hepatoma 3924A cells in tissue culture after 7 days of incubation. A concentration of 15 μM caused 50% inhibition of colony-forming ability (IC_{50}). Under the same conditions, hydroxyurea, also an inhibitor of ribonucleotide reductase, had an IC_{50} of 52 μM. Treatment for 1 hr with VF 122 of exponentially growing culture resulted in a biphasic exponential dose-response curve (D_0 = 0.5 mM, IC_{50} = 0.75 mM, and D_0 = 1.8 mM, IP = 0.8 mM). In plateau-phase cells, a threshold exponential curve was obtained (D_0 = 2.2 mM, D_q = 1.7 mM, and IC_{50} = 2.7 mM). Exponentially growing hepatoma 3924A cells were more sensitive to VF 122 than were plateau-phase cultures. In contrast, hydroxyurea killed only exponentially growing 3924A hepatoma cells, exhibiting an exponential plateau dose-response curve without achieving an IC_{50} value at concentrations from 1 to 200 mM. In synchronized cultures, VF 122 (1 mM for 1 hr) was toxic for cells in mid and late G_1 phase, in early and mid S phase, and, to a lesser degree, in G_2 phase. Hydroxyurea (10 mM for 1 hr) killed cells only in S phase. Proliferating and resting hepatoma 3924A cells recovered from sublethal and potentially lethal damage induced by VF 122. [Cancer Treat Rep 66:2071–2080, 1982]

Until recently, hydroxyurea (HU) was the main drug in clinical use with a primary target of ribonucleotide reductase (1–4). This neoplastic progression-linked key enzyme (5–7) catalyzes the reductive conversion of ribonucleotide to deoxyribonucleotide, leading to DNA synthesis and cell replication (8). Some newly synthesized derivatives of benzohydroxamic acid proved to be better inhibitors of the reductase activity and tumor growth than HU. Among them was 3,4,5-trihydroxybenzohydroxamic acid (VF 122), a hydroxy derivative of benzohydroxamic acid. VF 122 inhibited ribonucleotide reductase in vitro (10 μM caused 50% inhibition of colony-forming ability [IC_{50}]) and increased survival time of leukemic mice by 78% (9,10). A structure-activity relationship study indicated that the ring substituents play an important role in the inhibitory potency of hydroxamic acid (11). It was determined that 3,4-dihydroxybenzohydroxamic acid was the most potent derivative in this series (9,10), but recent work in this laboratory showed that according to IC_{50} values of colony-forming studies, VF 122 was five times stronger as a killing agent for rat hepatoma 3924A cells (J. Ban and G. Weber, unpublished observation).

The action of VF 122 was investigated to elucidate the mechanism of cytotoxicity on proliferating and resting rat hepatoma 3924A cells in tissue culture as measured by colony-forming ability. The lethality, as a function of VF 122 concentration and time exposure as well as of cell cycle-dependent toxicity, was studied and compared with results obtained with HU. In addition, the response of proliferating and resting tumor cells to drug-induced damage was examined.

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[1] Received June 1, 1982; revised Sept 9, 1982; accepted Sept 28, 1982.

[2] Supported by Public Health Service grants CA-13526 and CA-05034 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Laboratory for Experimental Oncology, Indiana University School of Medicine, Indianapolis.

[4] *Present address:* Central Institute for Tumors and Allied Diseases, Zagreb, Yugoslavia.

[5] *Present address:* Research Institute of Oncopathology, Budapest, Hungary.

**Reprint requests to:* George Weber, MD, Laboratory for Experimental Oncology, Indiana University School of Medicine, 1100 W Michigan St, Indianapolis, IN 46223.

MATERIALS AND METHODS

Cell culture.—Hepatoma 3924A cells were grown in monolayer cultures in McCoy's 5A medium with 10% fetal calf serum (Grand Island Biological Co, Grand Island, NY) and were incubated in a 5% CO_2:95% air atmosphere. To keep the cells in logarithmic growth, they were subcultured three times a week. For experiments on exponentially growing populations, 5×10^5 hepatoma 3924A cells were seeded into plastic flasks (surface area, 25 cm^2) and incubated for 48 hrs. For studies on plateau-phase cells, cultures were incubated for 96 hrs without changing the medium. Experimental methods, definitions, and cytogenetic characteristics of plateau and exponential phases have been reported elsewhere (12).

Synchronization technique.—Synchronized cell populations were used to study the cell cycle phase specificity of the drug. Synchrony was achieved by mitotic selection (13,14). The mitotic index was 90% as determined by counting metaphases after aceto-orcein staining in a hemocytometer. The length of the phases of the cell cycle was determined as reported previously (12). Current results indicated that the duration of each phase was as follows: G_1, 7 hrs; S, 4.5 hrs; and G_2, 2.5 hrs. The average cell cycle time was 14 hrs (15).

Dose-response experiments.—Exponential- or plateau-phase cells were treated with different concentrations of drugs. Cultures were exposed to VF 122 or HU for 1 hr, the drug was then removed, flasks were rinsed three times with phosphate-buffered saline (PBS) containing (in g/L) KCl (0.20), KH_2PO_4 (0.20), NaCl (8.00), and $Na_2HPO_4 \cdot 7H_2O$ (2.16). The cells were then trypsinized, counted with an electronic counter (Coulter Electronics, Inc, Hialeah, FL), and seeded into culture flasks (500 cells/25 cm^2). The pH was adjusted with 5% CO_2 in air. After 7 days of incubation, the medium was removed and the cells were stained with a saturated solution of crystal violet in 0.85% sodium chloride (12). Colonies containing > 50 cells were counted and results were expressed as a percent of colonies of untreated cells. To avoid growth stimulation, plateau-phase cells were treated by adding the drugs (prepared in PBS) to the exhausted medium for 1 hr; cells were then trypsinized and plated as described above. Long-term experiments were performed with proliferating cultures. Cells were seeded (500/flask) and exposed to the drug for 7 days, as reported elsewhere (15). Colonies were stained and counted as above. The plating efficiencies of untreated control cultures of exponentially growing cells, plateau-phase cells, and mitotically selected cells were 76%, 72%, and 50%, respectively. Mean, standard error, and statistical evaluation by the t test were calculated for each dose or time point. Differences between mean values yielding a probability of < 5% were considered significant.

Recovery from sublethal damage.—As a test for recovery from sublethal damage, fractionated doses were used. The treatment was fractionated using the total dose concept of drug concentration and duration of treatment (C × T) (16). Exponential- or plateau-phase cells received the total dose in two equal sequential exposures. All cells received one-half of the total dose of VF 122 at 0 hr. At the end of the first treatment (30 mins), one group of cells was immediately given the same dose, thus allowing no time between treatments for recovery. The other groups of cells were washed and reincubated in fresh or in depleted plateau-phase medium, depending on the cell growth phase; they were exposed for 30 mins to the second half of the total dose 1–6 hrs later. All cells were plated for colony formation immediately after exposure to the second dose of the drug. Controls obtained at regular intervals consisted of cells given either one-half dose or the total dose (for details *see* figs 5 and 6).

Recovery from potentially lethal damage.—Exponential- or plateau-phase cells were treated with different concentrations of VF 122 for 30 or 60 mins. Following treatment, cells were plated immediately for colony formation or were washed three times with PBS and incubated at 37°C in depleted plateau-phase culture medium (96 hrs old) for 2, 4, or 6 hrs before being plated for colony formation.

Drugs.—VF 122 was obtained from Dr. H. L. Elford, College of William and Mary, Newport News, VA. HU was purchased from Sigma Chemical Co, St. Louis. Drugs were stored under refrigeration and prepared with sterile PBS before use.

RESULTS AND DISCUSSION

Dose-Response Curves of VF 122 and Hepatoma 3924A Cells

The threshold-exponential dose-response survival curve of proliferating cells exposed to VF 122 for 7 days yielded an IC_{50} of 15 μM (fig 1). The mean lethal dose (D_0, the concentration required to reduce survival by 63% on the exponential part of the survival curve) was 12 μM, and the quasi-threshold dose [D_q, equal to the intercept with the abscissa of the exponential part of the survival curve (17)] was 6 μM. A dose-response curve for rat hepatoma 3924A cells exposed to HU for 7 days is presented in figure 1 for comparison. A threshold-exponential type curve with a wide shoulder yielded an IC_{50} of 52 μM, a D_0 of 46 μM, and a D_q of 43 μM. According to IC_{50} values, VF 122 was 3.5 times more active than HU after long-term treatment.

Study of Growth Phase Sensitivity to VF 122

To determine whether there was a growth phase-dependent sensitivity to VF 122, exponentially growing

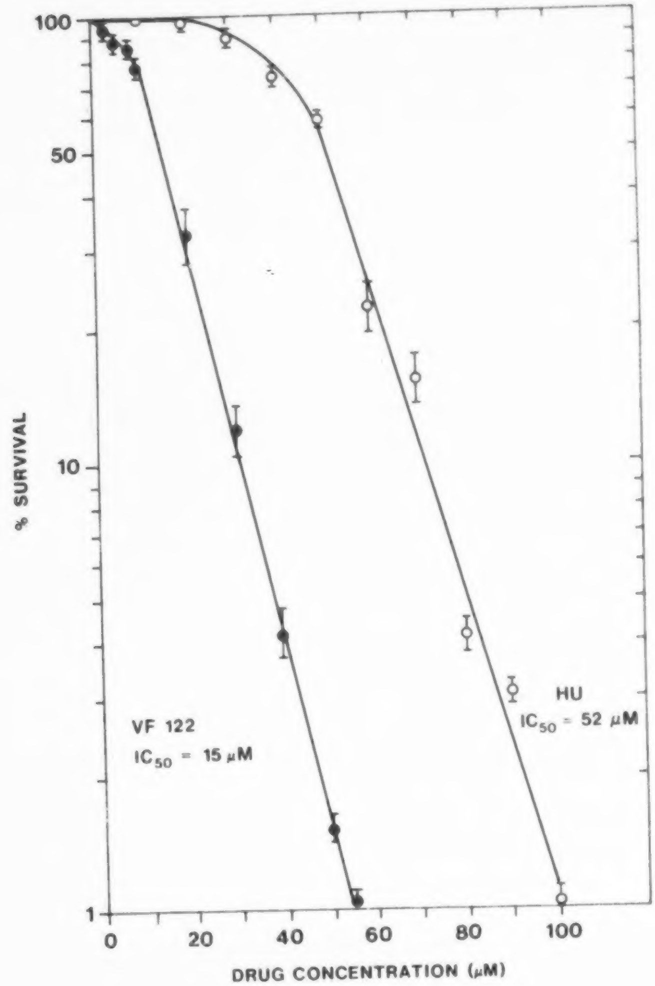

FIGURE 1.—Survival of proliferating hepatoma 3924A cells exposed to VF 122 or HU for 7 days. Cells were seeded (500/flask), supplemented with drugs, and incubated for 7 days. Colonies were counted, and counts were expressed as % of colonies of untreated cells. Each point is the average of triplicate samples of 3 experiments.

and plateau-phase cells were treated with the drug for 1 hr (fig 2). The treatment of exponentially growing cells resulted in a biphasic exponential dose-response curve with a D_0 of 0.5 mM for the initial part of the curve (IC_{50} = 0.75 mM) and a D_0 of 1.8 mM for the second part of the curve. The inflection point (IP), the concentration that eradicates the most sensitive cells from the population (17), was 0.8 mM. Plateau-phase cells were characterized by a threshold exponential curve with a moderately large shoulder region and exponentially decreased survival as a function of increasing concentrations (D_0 = 2.2 mM, D_q = 1.7 mM, and IC_{50} = 2.7 mM). Exponentially growing cells were more sensitive to VF 122 than plateau-phase cultures. This difference diminished when higher drug concentrations ($\geq$ 6 mM) were used (data not shown). These observations indicated that VF 122 could be a cycle-specific agent according to the kinetic classification of antitumor agents (18,19).

Since VF 122 revealed significant lethal effect against exponential- and plateau-phase cells, the data suggested that the drug might be effective not only for rapidly growing tumors but also for slowly growing solid tumors containing large fractions of resting cells. In

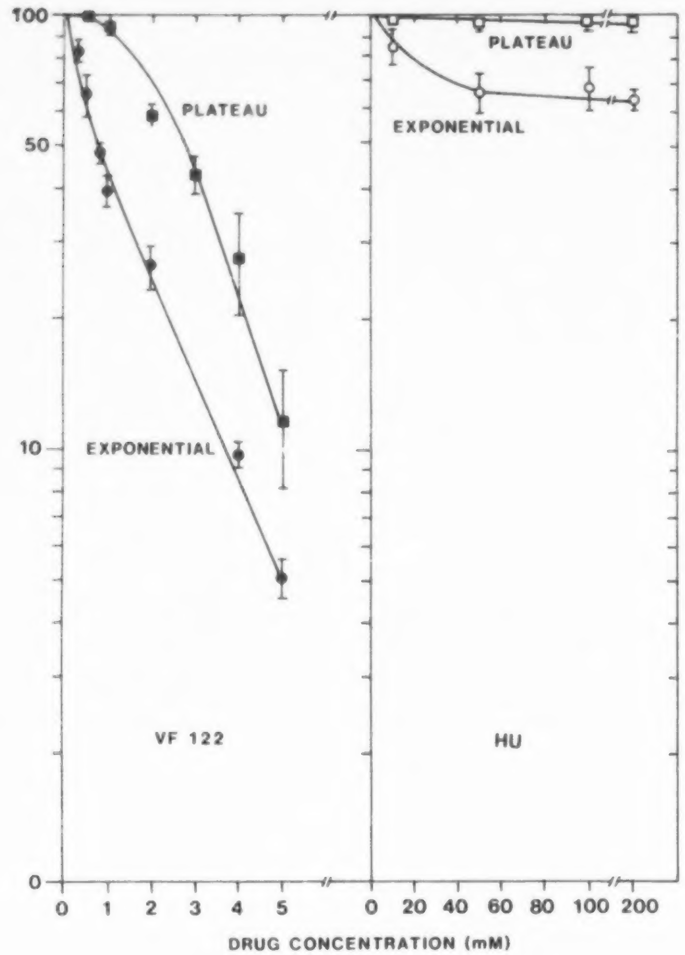

FIGURE 2.—Survival of hepatoma 3924A cells in exponential and plateau phases exposed to increasing concentrations of VF 122 and HU. Cells were treated for 1 hr, seeded, and counted after 7 days of incubation. Results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

contrast, HU did not kill the plateau-phase hepatoma 3924A cells (fig 2). Exponentially growing cells showed an exponential plateau dose-response survival curve that was characterized by an initial exponential decrease in survival (D_0 = 80 mM). A concentration of 50 mM killed 35% of the proliferating cells and then the surviving fraction leveled off, with no increased killing at doses as high as 200 mM. These results agreed with earlier observations on the action of HU on other cell lines (20–24).

Action of VF 122 in the Cell Cycle

It was reported previously (15,25) that cycle-specific agents produce significant fluctuations in age responses of synchronized cells; thus, it seemed desirable to compare the sensitivity of synchronized cells in different phases of the cell cycle (12–14). The position of cells in the cell cycle time was determined by pulse-labeling autoradiography (^{3}H-thymidine, 1.0 μCi/ml; specific activity, 2 Ci/mmol). Synchronized cultures were treated at regular intervals for 1 hr with 0.25 and 1 mM VF 122. This treatment killed 10% and 50%, respectively, of the cells in the asynchronous cell population, as determined by colony counts.

A concentration of 0.25 mM killed the cells chiefly in late G_1 and early S phase. The toxicity was expressed to a lesser degree in the early G_1 and late S phases.

There was only slight killing action on cells in other phases of the cycle (fig 3). The higher concentration of VF 122 (1 mM) was toxic also in mid G_1, mid S, and G_2 phases. Cells in the M phase were virtually insensitive to VF 122. The cells in G_1–S transition were five times more sensitive than those in G_2 phase. The greater sensitivity of cells in the G_1–S transition region could be explained by different sensitivity of the cell populations to the drug, as shown in figure 2, where the dose-survival curve of exponential cells had two different slopes. Inhibition of cell growth in S phase may also be attributed to an action of VF 122 on DNA synthesis, as measured in HeLa cells (H. L. Elford, personal communication).

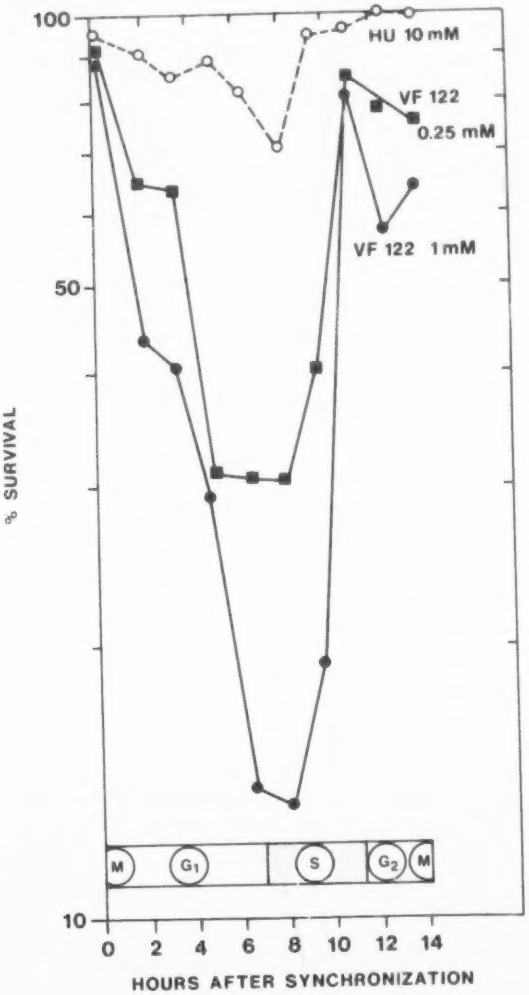

FIGURE 3.—Sensitivity of synchronized hepatoma 3924A cells to VF 122 (0.25 and 1 mM) and HU (10 mM). Synchronized cells (1000/flask) were exposed to drug at regular intervals after mitotic selection. Drug was removed after 1 hr, cells were washed with PBS, and fresh medium was added. Synchronized, untreated controls were also plated for each time point. Results were expressed as % of colonies of untreated cells and were evaluated as in fig 1. Each point is the average of triplicate samples of 2 experiments.

HU (10 mM for 1 hr) showed clear-cut phase-specific toxicity, killing cells in S phase only (survival was 70%; fig 3). This agreed with findings in other cell lines (24,26).

Stability of Biologic Activity of VF 122

It was important to determine the stability of VF 122 in cell culture because of the length of some of the experiments. Exponentially growing cells were exposed to 0.5 and 1 mM VF 122 for increasing periods of time. Biphasic time-dependent survival curves are presented in figure 4.

Here again, as in the dose-survival curve of proliferating cells (fig 2), two different slopes are evident. The final slopes appearing within 30–60 mins after VF 122 treatment manifested different time-dependent action of the drug, depending also on the concentration.

To examine the biologic stability of VF 122 in different solutions, the drug was dissolved and preincubated at 37°C in complete medium with serum and in PBS for different periods (up to 48 hrs). The drug solutions were then added to proliferating cells, cultures were incubated for 7 days, and plating efficiencies were compared. Since no difference in the cell survival was found, it was assumed that the drug was stable under these conditions (data not shown).

Response of Exponential- and Plateau-Phase Cells to Sublethal Damage

The shape of the dose-response survival curve of plateau-phase cells suggested their capacity to accumulate some damage without lethality, whereas no shoulder region was observed for the dose-response curve of proliferating cells. To test the recovery from sublethal damage (SLD), the treatments were fractionated using the integral dose concept (16), and fractions of the total dose, given sequentially, were used to treat proliferating and resting cells.

Survival of exponential cells that received two doses of 1 mM VF 122 for 30 mins, separated by 0, 1, 2, 4, 6, or 8 hrs, is shown in figure 5. The theoretical value for survival after two treatments was defined as the survival of cells that completed repair of SLD from the first dose, before the second dose was given (16). Therefore, the theoretical value for survival (S) after two treatments is $(\%S)_1 \times (\%S)_2/100$. If the surviving cells have complete repair of SLD from the first dose they should survive a second dose by the same fraction. The survival of exponentially growing hepatoma 3924A cells treated with a single dose of VF 122 (1 mM for 30 mins)

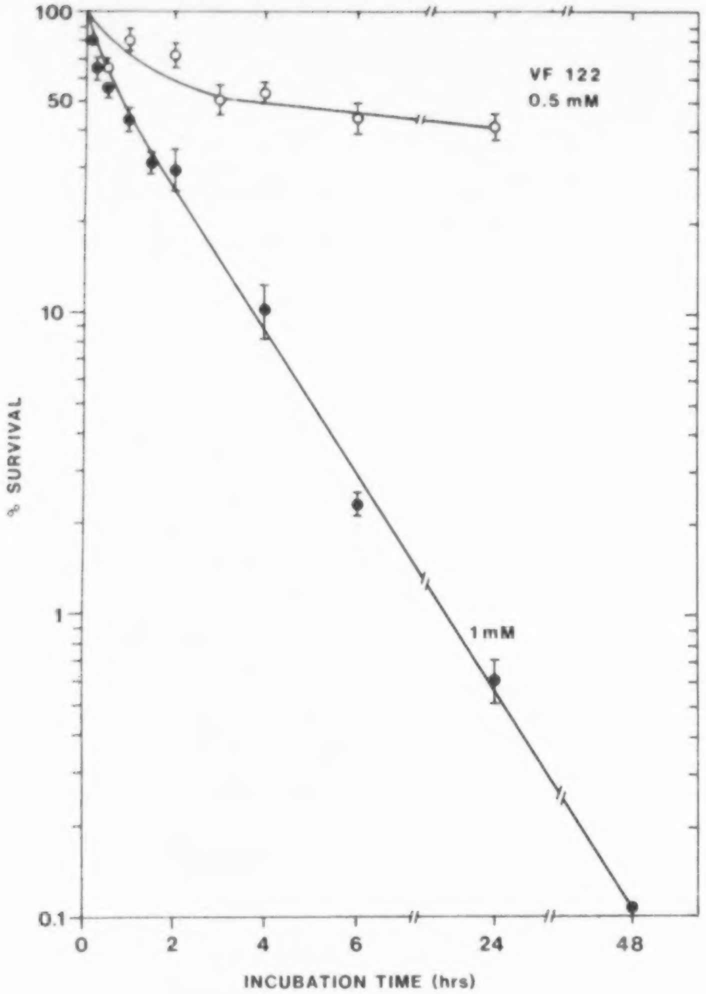

FIGURE 4.—Survival of proliferating hepatoma 3924A cells exposed to VF 122 as a function of time. Exponentially growing cells were treated with 0.5 or 1 mM VF 122 and then seeded for survival at regular intervals. Results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

was 57%; therefore, the theoretical value for survival was 32%. Figure 5 shows that recovery was observed even after 2 hrs; the 37% survival at this time point was nearly the same as the theoretical value. With an interval of 6 hrs between the doses, survival was 60%, which was 1.8 times higher than the theoretical value. In fact, this survival was nearly equal to that determined at zero time for the cells treated with one-half of the total dose (1 mM for 30 mins); it was also higher than survival, determined at zero time, of the cells that received the total dose at one time (2 mM for 30 mins or 1 mM for 60 mins).

Plateau-phase cells were also treated with fractionated doses. However, higher concentrations were given because of the lower sensitivity of these cells to VF 122. Between the two doses, cells were maintained in the medium from plateau-phase cultures to avoid growth stimulation. Figure 6 shows that plateau-phase cells recovered from SLD induced by VF 122 (3 mM for 30 mins, spaced in time). The survival of cells that received fractionated doses after 6 hrs of incubation was 1.8 times higher than the theoretical survival (30%), indicating

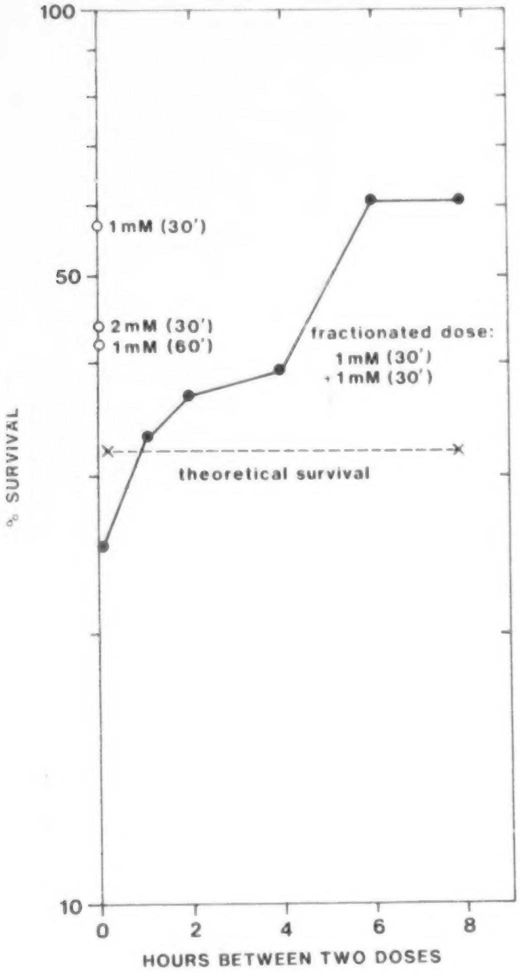

FIGURE 5.—Survival of exponentially growing hepatoma 3924A cells after total (2 mM for 30 mins or 1 mM for 60 mins), $^1/_2$ of total (1 mM for 30 mins), and fractionated-dose treatment with VF 122 (●). Cells that received fractionated doses were exposed to a single dose of 1 mM VF 122 for 30 mins at 0 time. 1 group of cells (0 time point) was treated immediately with same dose, with no time interval. Other groups of cells were washed with PBS and reincubated in fresh medium. At times indicated by points, cells were given same additional dose (1 mM for 30 mins) and were plated immediately for colony formation. Colonies were counted and results were evaluated as in fig 1. Each point is the average of triplicate samples of 3 experiments.

that resting cells also recovered from VF 122-induced damage.

Response of Exponential- and Plateau-Phase Cells to Potentially Lethal Damage

Since recovery from potentially lethal damage (PLD) could contribute to SLD recovery during the experiments designed to test for SLD, it was relevant to determine the response to PLD. The recovery from PLD was defined as the increase in survival of cells that were exposed to suboptimal growth conditions after a single drug treatment (16,27,28). Exponentially growing hepatoma 3924A cells were treated with a single dose of 1 or 2 mM VF 122 for 60 mins and were plated immediately for colony counts (survival rates were 42% and 25%, respectively). Another set of samples was given the

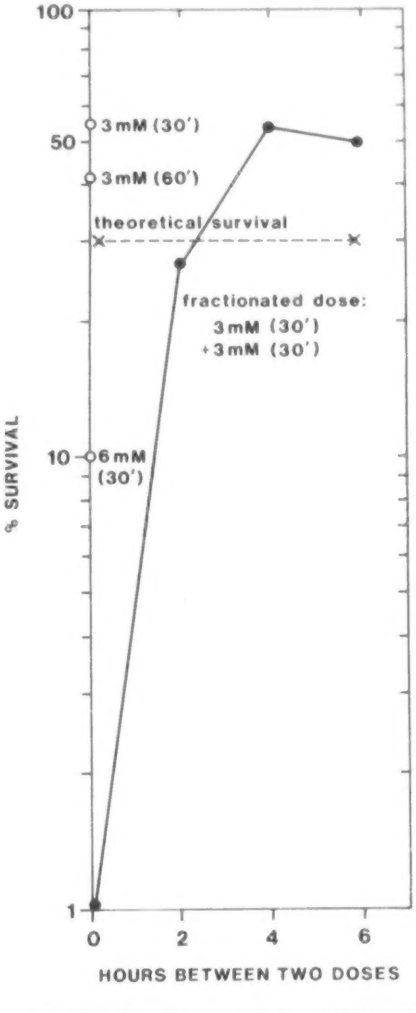

FIGURE 6.—Survival of plateau-phase hepatoma 3924A cells after total (6 mM for 30 mins or 3 mM for 60 mins), $^1/_2$ of total (3 mM for 30 mins), and fractionated-dose treatment with VF 122 (●). Cells that received fractionated doses were exposed to a single dose of 3 mM VF 122 for 30 mins at 0 time. During the interval between receiving 2 doses, cells were incubated in depleted plateau-phase medium. Other experimental conditions and evaluations of results were as in fig 5. Each point is the average of triplicate samples of 3 experiments.

Table 1.—Recovery of plateau-phase hepatoma 3924A cells from PLD

Treatment at 0 time		% survival after incubation* for—			
VF 122 (mM)	Incubation (mins)	0 hrs	2 hrs	4 hrs	6 hrs
3.0	30	55	58	66†	76†
3.0	60	42	59†	73†	80†
6.0	30	10	25†	34†	52†
6.0	60	2	77†	53†	75†

*Cells were incubated in depleted 96-hr plateau-phase medium for increasing time periods. Mean values of triplicate determinations are given.

†Significantly higher survival compared to cells plated immediately (0 time) ($P < 0.05$).

same drug treatment; after removal of the drug, the cells were incubated in depleted plateau-phase medium for different periods before seeding for colony formation. After 6 hrs of incubation, the survival values were significantly higher (80% for 1 mM VF 122 and 63% for 2 mM VF 122) than those of cells plated immediately after the treatment. These results clearly indicated the ability of exponentially growing cells to recover from PLD. Plateau-phase cells were treated and assayed in the same manner; they exhibited similar ability to repair PLD. These data are presented in table 1.

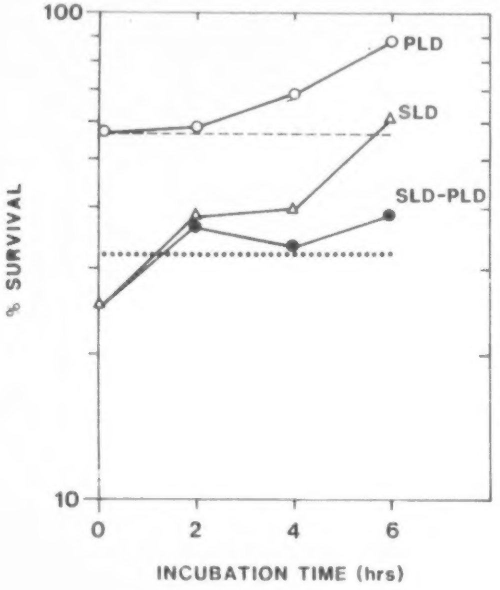

Figure 7.—Real recovery of exponentially growing hepatoma 3924A cells from SLD. Cells were exposed to fractionated doses of VF 122 (1 mM for 30 mins) to obtain SLD curve described in fig 5. To measure PLD, cells were treated with 1 mM VF 122 for 30 mins at 0 time. 1 group of cells was plated immediately for colony formation. Other groups of cells were washed with PBS and reincubated in fresh medium. At times indicated, cells were plated for colony formation. SLD-PLD curve was obtained by reducing SLD survival points (60% after 6-hr incubation) by the fraction due to recovery from PLD. This fraction was calculated as the difference between PLD survival points obtained after incubation in fresh medium (87% for 6 hrs incubation) and value obtained without incubation (57% survival, indicated by ----). Colonies were counted and results were evaluated as in fig 1. Theoretical survival (·····).

Parallel recovery experiments were also designed to determine the contribution made by PLD recovery to recovery from SLD. For that purpose, exponentially growing cells were treated as for PLD experiments (1 mM VF 122 for 30 mins), but the cells were incubated in fresh medium for 2, 4, or 6 hrs before seeding for colony formation (fig 7). To determine the contribution made by PLD recovery, an SLD-PLD curve was constructed by reducing the SLD survival points by the survival fraction due to recovery from PLD. The fraction that recovered from PLD was calculated by subtracting the value obtained at zero time (no incubation in fresh medium) from the survival values on the PLD curve obtained after incubation for 1–6 hrs (fig 7). The real recovery from fractionated doses of VF 122 is given in figure 7 for exponentially growing cells and in figure 8 for plateau-phase cells.

All these data suggested that part of the observed recovery from SLD was due to recovery from PLD. PLD recovery was high enough to mask the true response to SLD. These data support the observation of Barranco et al (16) that one type of damage (PLD) could be so great as to mask the true response of cells to another type of damage (SLD). Different sensitivities to VF 122 observed between proliferating and resting hepatoma 3924A cells could not be explained with survival responses to SLD. Namely, exponentially growing cells treated with VF 122 did not exhibit a shoulder-like dose-survival curve but they recovered from SLD as well as did plateau-phase cells.

The results presented here might also contribute to the study of the regrowth of tumor cells in in vitro systems, ie, a tissue culture system that is based on a transition from the nonproliferating to the proliferating phase is useful in the study of the neoplastic growth program (29,30).

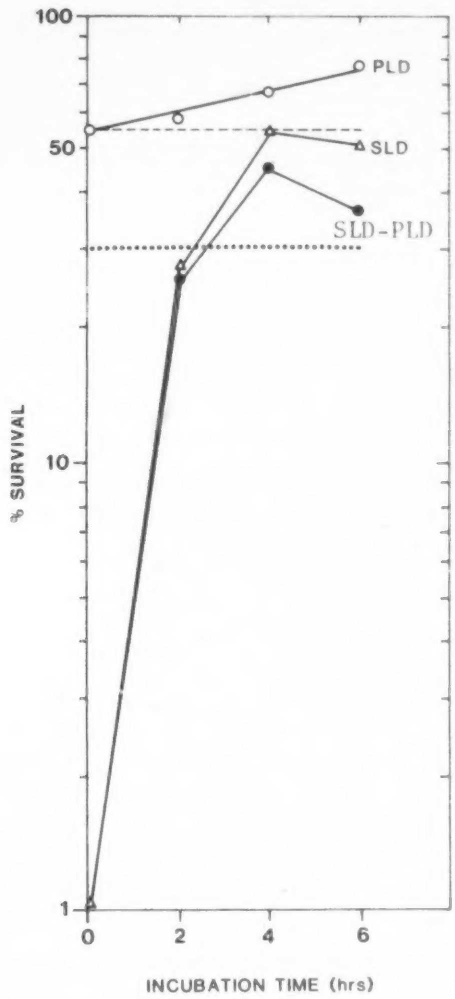

FIGURE 8.—Real recovery of plateau-phase hepatoma 3924A cells from SLD. Cells were exposed to fractionated doses of VF 122 (3 mM for 30 mins) to obtain SLD curve described in fig 6. To measure PLD, cells were treated with 3 mM VF 122 for 30 mins at 0 time. 1 group of cells was plated immediately for colony formation. Other groups of cells were washed with PBS and reincubated in depleted plateau-phase medium. At times indicated, cells were plated for colony formation. Colonies were counted and results were evaluated as in fig 1. Theoretical survival (·····); no incubation in depleted plateau-phase medium(----).

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Prednimustine in Combination With Methotrexate and 5-FU (PMF): A Pilot Study[1]

Else Boesen and H. T. Mouridsen[2,*]

A group of 47 patients with advanced breast cancer were treated with a combination of prednimustine, methotrexate, 5-FU, and tamoxifen. In this combination, with methotrexate and 5-FU administered at standard doses, hematologic toxicity of prednimustine at a dose of 100 mg/m² orally on Days 1–5 repeated every 4 weeks was acceptable. Nausea and vomiting occurred in 40 of the 47 patients but was only grade 1–2 in 35 of these patients. Only one patient had alopecia to the extent of requiring a wig. Response to treatment could be evaluated in 28 patients; 21 achieved a response (complete or partial) lasting a median of 13 months. [Cancer Treat Rep 66:2081–2083, 1982]

Prednimustine is an ester of chlorambucil and prednisolone. In a previous phase II study an intermittent schedule of prednimustine proved to be active in advanced breast cancer (1), and in a recent phase III study both intermittent and continuous schedules of prednimustine were demonstrated to be superior to continuous treatment with the combination of chlorambucil plus prednisolone (2). CMF (cyclophosphamide, methotrexate, and 5-FU) has been one of the most widely used combination therapies in advanced breast cancer, with a response rate of about 50% (3,4), and recent studies have indicated that the response rate is further increased when tamoxifen is added to the combination (5,6).

The obvious objective of studies in advanced breast cancer is to develop regimens with higher efficacy and/or lower toxicity. Based on the available data in breast cancer it seems that prednimustine may be at least as effective as cyclophosphamide and that it probably causes less subjective toxicity and a lower grade of alopecia (2). Therefore, a randomized trial to compare CMF with a combination of prednimustine, methotrexate, and 5-FU (PMF) seems reasonable. However, preliminary experience with PMF is first needed.

The objectives of the present study were primarily to analyze the tolerability of prednimustine administered at different dose levels in combination with methotrexate, 5-FU, and tamoxifen, and secondarily to analyze the efficacy of this combination.

MATERIALS AND METHODS

Patients.—Postmenopausal patients $\leq$ 68 years of age with advanced progressive breast cancer, with or without measurable or evaluable lesions, were entered in the trial, provided they had not received prior cytotoxic therapy, had normal bone marrow function as defined by a wbc count $> 4000/\mu l$ and a platelet count $\geq 100{,}000/\mu l$, had no other previous or concomitant malignant disease, and had no glucosuria or symptoms of active gastric or duodenal ulcer. Oral informed consent was obtained.

Treatments.—Two consecutive series (1 and 2) of patients who fulfilled the criteria of eligibility were treated with PMF. Patients in series 1 received the following doses per cycle: prednimustine, 80 mg/m² orally on Days 1–5; methotrexate, 40 mg/m² iv on Days 1 and 8; and 5-FU, 600 mg/m² iv on Days 1 and 8. Patients in series 2 received 100 mg/m² of prednimustine orally on Days 1–5 and doses of methotrexate and 5-FU as in series 1. In both series the cycles were repeated every 4 weeks. In addition, all but four patients received 20 mg of tamoxifen twice daily.

Assessment of hematologic toxicity and adjustment of dose.—During the first three cycles wbc and platelet counts were measured weekly. Thereafter, they were measured only on Days 1 and 8 of each cycle. Doses were adjusted for hematologic toxicity on Days 1 and 8 of each cycle according to the following values for wbc

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[1] Received Mar 8, 1982; revised Aug 26, 1982; accepted Sept 22, 1982.

[2] Department of Oncology A, Finsen Institute, Copenhagen, Denmark.

**Reprint requests to:* H. T. Mouridsen, MD, Department of Oncology A, Finsen Institute, 49 Strandboulevarden, DK-2100 Copenhagen Ø, Denmark.

TABLE 1.—Wbc and platelet count nadirs in patients given PMF in series 1 and 2*

Toxicity grade†	No. of patients in series 1 or 2											
	Cycle 1				Cycle 2				Cycle 3			
	Wbc count		Platelet count		Wbc count		Platelet count		Wbc count		Platelet count	
	1	2	1	2	1	2	1	2	1	2	1	2
1	18	12	23	18	11	7	23	18	9	6	18	18
2	6	7	2	1	10	9	1	0	9	10	2	1
3	1	0	0	1	4	3	1	1	3	3	1	0
4	1	2	1	1	1	1	1	1	0	0	0	0

*Series 1 = 26 patients; prednimustine dose, 80 mg/m^2. Series 2 = 21 patients; prednimustine dose, 100 mg/m^2.

†Toxicity grades according to wbc and platelet count, respectively ($\times 10^3/\mu l$): 1, ≥ 3.0 and ≥ 100; 2, 2.0–2.9 and/or 75–99; 3, 1.0–1.9 and/or 50–74; and 4, < 1.0 and/or < 50.

($\times 10^3/\mu l$) and platelet ($\times 10^3/\mu l$) counts: ≥ 3 and ≥ 100, 100% of dose; 2.0–2.9 and/or 75–99, 50% of dose; 1.0–1.9 and/or 50–74, 25% of dose; and < 1.0 and/or < 50, no drug.

Assessment of nonhematologic toxic effects.—The nonhematologic toxic effects were graded 0–4, 0 indicating absent, 1 indicating mild, 2 indicating moderate, 3 indicating severe, and 4 indicating life-threatening toxicity according to World Health Organization criteria (7).

Assessment of response to treatment.—Response to treatment was analyzed, according to the International Union Against Cancer criteria (8), in patients with measurable and/or evaluable lesions who had received a minimum of two cycles.

RESULTS

From June 1979 to April 1980 a total of 47 patients entered the trial, 26 in series 1 and 21 in series 2.

Hematologic toxicity and dose adjustment.—The wbc and platelet count nadirs during the first three cycles in series 1 and 2 are given in table 1. It appears that the wbc counts were depressed significantly more often than platelet counts, and it further appears that during the first three cycles 70% of the patients had a wbc count nadir corresponding to toxicity grade 2 or more. In both series, the doses of prednimustine, methotrexate, and 5-FU had to be reduced because of toxic effects, more so in series 2 (table 2). A total of 38 patients were treated for an additional 1–19 months (median, 10.5). No signs of cumulative hematologic toxicity were observed in these patients, and median drug doses in cycles 5, 10, and 15 were 74%, 77%, and 72%, respectively. Three patients developed severe leukopenia and sepsis requiring admission to hospital and treatment with antibiotics.

Nonhematologic toxic effects.—Nausea and vomiting occurred in 40 of the 47 patients; five patients had grade 3–4 and 35 had grade 1–2. Only one of the patients developed alopecia to the extent of requiring a wig. One patient developed extensive condylomata during treatment and another developed upper abdominal pain which subsided on further treatment when cyclophosphamide was substituted for prednimustine. Gastroscopy in this patient was normal.

Response to treatment.—Response could not be analyzed in 19 patients. Ten patients did not have measurable or evaluable disease. Another four patients did not receive tamoxifen, two received only one cycle, one was lost to follow-up, and two had a previously treated carcinoma of the cervix but without signs of recurrence of that disease. In these 19 patients we analyzed only the time until progression. Among the other 28 patients with evaluable disease (16 in series 1 and 12 in series 2), complete remission was observed in 12 cases and partial remission in nine cases, giving a total remission rate of 75% with a median duration of response of 13 months. No change was observed in five patients, and progressive disease was seen in two patients. Among the nine responding patients in series 1, the dominant sites of lesion were: soft tissue (six patients), viscera

TABLE 2.—Average relative drug doses

Series	% of dose					
	Cycle 1		Cycle 2		Cycle 3	
	Day 1	Day 8	Day 1	Day 8	Day 1	Day 8
1	98	74	83	75	84	79
2	94	83	84	69	76	69

(two), and bone (one). The dominant sites in the 12 responding patients in series 2 were: soft tissue (four), viscera (five), and bone (three). The median time until progression was 11 months in the 28 patients with evaluable disease, compared to 8 months in the 19 patients with unevaluable disease.

DISCUSSION

The major objective of the present study was to analyze the toxicity of prednimustine when combined with methotrexate and 5-FU. In the PMF combination, with methotrexate and 5-FU administered at standard doses, hematologic toxicity of prednimustine at a dose of 100 mg/m^2 on Days 1–5 was acceptable. Gastrointestinal symptoms were less pronounced with PMF than normally reported with CMF (6), and only one of the 47 patients developed alopecia to the extent of requiring a wig, which definitely seems to be less frequent than with the CMF combination.

Response to treatment could be evaluated in 28 patients, 21 (75%) of whom responded with a 13-month median duration of response. These figures are in agreement with results achieved with the CMF plus tamoxifen combination (6).

In conclusion, it seems that prednimustine can be safely used in combination with methotrexate and 5-FU. The frequency of alopecia is definitely lower with PMF than with CMF. Conclusions as to whether this relates also to subjective side effects will require a randomized study, as will a final conclusion concerning the efficacy of PMF compared to that of CMF.

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BRIEF REPORTS

Cellular and Plasma Kinetics of Daunorubicin Given by Two Methods of Administration in a Patient With Acute Leukemia[1,2]

Michael W. DeGregorio,* Carlos J. Carrera, John C. Klock, Charles H. Pegelow, and Jordan R. Wilbur [3,4]

Leukemic cell and plasma concentrations of daunorubicin were examined serially in a patient with acute lymphoblastic leukemia following administration of the drug by two different regimens: regimen A—an iv bolus dose of 50 mg/m^2, and regimen B—a loading dose of 15 mg/m^2 followed by 35 mg/m^2 infused over 4 hours. Peak plasma levels were four times higher after regimen A than B, whereas leukemic cell concentrations were not significantly different. No difference in acute toxicity or therapeutic efficacy was seen after either method of administration. However, we have demonstrated that peak plasma levels of daunorubicin can be reduced by continuous infusion without significantly altering target tissue concentrations. [Cancer Treat Rep 66:2085–2088, 1982]

The anthracycline antibiotics doxorubicin (DOX) and daunorubicin (DNR) are among the most important chemotherapeutic agents currently used in the treatment of patients with acute leukemia (1). These drugs are usually administered by a bolus dose over 3–5 minutes. In addition to the toxic effects common to most cancer drugs (bone marrow suppression, alopecia, stomatitis, nausea, and vomiting), the effective use of anthracyclines is limited by the unique toxicity of cardiomyopathy related to the cumulative dose of these drugs (2,3). Patients who could benefit from the continued use of anthracycline therapy may not be able to receive this treatment because of either the risk or actual development of life-threatening cardiac disease. Two studies have shown that altering the dosage schedules of DOX has reduced the incidence of cardiotoxicity and has allowed higher cumulative doses (4,5). The lowering of peak plasma levels may be one factor responsible for the reduction of DOX-induced cardiotoxicity (5).

We examined the cellular and plasma kinetics of DNR in a patient with acute leukemia after a bolus dose and again after a small iv loading dose followed by a 4-hour continuous infusion. The purpose of this preliminary study was to determine whether altering the method of administration would affect the cellular and plasma kinetics of DNR.

Patient History

The patient is a 26-year-old white male with acute lymphoblastic leukemia in his third relapse who had normal liver and renal functions (creatinine < 1.3 mg/100 ml) during the study periods. Past induction regimens included vincristine, prednisone, L-asparaginase, DNR, and cytarabine. At this time, remission induction was attempted using a combination of cyclophosphamide, vincristine, DNR, and prednisone. To avoid possible drug-drug interaction, cyclophosphamide was not administered within 24 hours of the DNR doses. The total cumulative dose of DNR was 225 mg/m^2 before bolus therapy and 275 mg/m^2 before the small bolus followed by short continuous infusion. Just prior to the bolus dose of 50 mg/m^2 of DNR, the peripheral blast

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[1] Received Apr 15, 1982; revised Sept 14, 1982; accepted Sept 28, 1982.

[2] Supported by the Louis R. Lurie Foundation, the Kent Bourne Memorial Fund, University of California Cancer Research Coordinating Committee account, and the American Society of Hospital Pharmacist Research and Education Foundation Grant.

[3] Children's Cancer Research Institute (*M. W. DeGregorio, C. H. Pegelow,* and *J. R. Wilbur*), Institute of Cancer Research (*M. W. DeGregorio* and *J. C. Klock*), Pacific Medical Center, and the Cancer Research Institute, University of California (*M. W. DeGregorio* and *C. J. Carrera*), San Francisco.

[4] We thank Maria S. Drake for technical assistance and James Harris for typing this manuscript.

**Reprint requests to:* Dr. Michael DeGregorio, Children's Cancer Research Institute, 2351 Clay St, S-512, Pacific Medical Center, San Francisco, CA 94115.

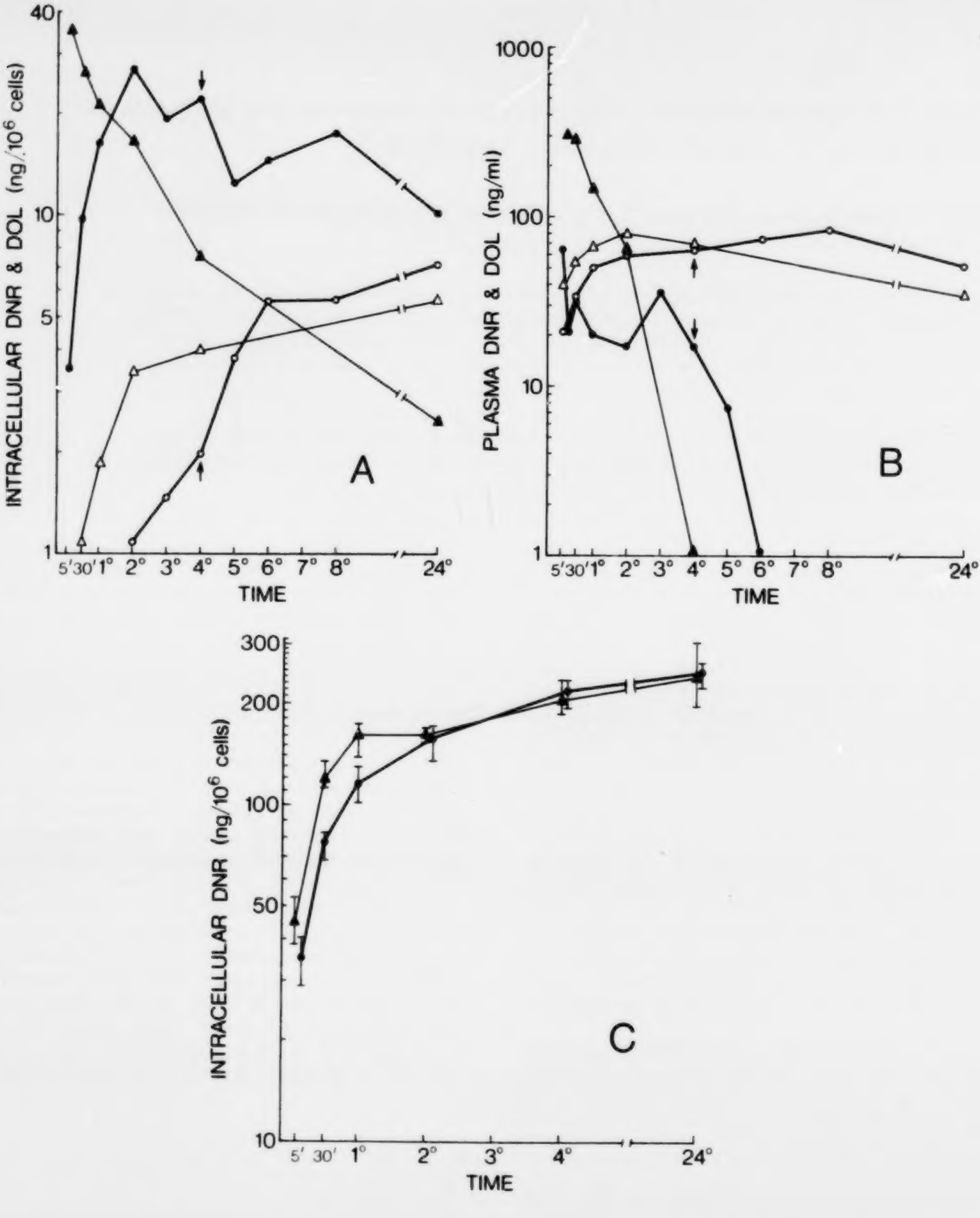

FIGURE 1.—Leukemic cell (A) and plasma (B) concentrations of DNR (▲) and DOL (△) after bolus administration, and DNR (●) and DOL (○) during and after the 4-hr infusion. Arrows indicate end of infusion. C. In vitro uptake of DNR by leukemic cells before bolus injection (▲) and before continuous infusion (●).

count was 134,000/mm^3. The peripheral blast count nadir 7 days after therapy was 37,000/mm^3 and thereafter quickly rose to 74,000/mm^3. A second course of cyclophosphamide, vincristine, DNR, and prednisone was instituted in the same manner with the exception that DNR was administered by a loading dose of 15 mg/m^2 followed by a 35-mg/m^2 infusion over 4 hours. This resulted in a decrease of the peripheral blast count to a nadir of 18,000/mm^3 8 days after therapy.

MATERIALS AND METHODS

Blood samples were anticoagulated with EDTA at several time points and rapidly cooled to 4°C. Each sample was centrifuged at 200 × *g* for 10–15 minutes and plasma was removed. The resulting plasma was centrifuged a second time at 1040 × *g* for 5–10 minutes to remove remaining leukocytes. The buffy coat was depleted of erythrocytes by cold hypotonic lysis. Remaining leukocytes (> 98% lymphoblasts) were then resuspended in 2 ml of isotonic saline and the cell concentration was determined by a Coulter counter. Samples (1-ml) of plasma and cell suspensions were then processed for higher performance liquid chromatography (HPLC) analysis.

Quantitation of leukemic cell and plasma concentration of DNR was performed utilizing HPLC methods previously reported (6). To summarize: an internal standard DOX was added to plasma and cell samples which were then extracted using a chloroform/methanol mixture. Extracted samples were stored at –20°C in the dark until the day of analysis. Samples were reconstituted with 100 µl of chloroform and 25-µl aliquots were injected for analysis.

In vitro uptake and metabolism of DNR by the patient's leukemic cells were determined as previously described (7). To summarize: leukemic cells before each treatment were isolated from peripheral blood using Ficoll-Hypaque density gradient centrifugation. The viability was assessed before and after each experiment by erythrosin-B dye exclusion. Leukemic cells suspended in RPMI-1640 medium enriched with 15% fetal calf serum were exposed to ^{14}C-radiolabeled DNR at a test concentration of 1 µg/ml. Cells were incubated at 37°C and at several time points triplicate samples were assayed for intracellular DNR concentration by liquid scintillation spectrometry.

RESULTS

The cellular and plasma concentrations of DNR and daunorubicinol (DOL) resulting from the two methods of drug administration are shown in figure 1, A and B. Plasma concentrations of DNR declined rapidly following both the bolus dose and the end of the infusion. The peak plasma concentrations of DNR during infusion were approximately 20%–25% of the peak achieved after the bolus dose. Intracellular DNR levels after the bolus dose also declined rapidly, whereas the leukemic cell levels of DNR increased during the period of infusion and actually exceeded intracellular levels achieved after the bolus dose. Plasma and leukemic cell concentrations of DOL were not significantly different after either method of administration.

The in vitro uptake of DNR by the leukemic cells before each treatment is shown in figure 1C. No significant difference in the pattern of in vitro uptake was seen. The in vitro metabolism of DNR as determined by HPLC techniques was not significant (< 5%) in either study after 24-hour incubation with 1 µg/ml.

DISCUSSION

Our results show that compared to bolus therapy, the administration of DNR by short continuous infusion reduces peak plasma levels but achieves similar drug concentrations in the leukemic cells.

Experimental data in animal models suggest that the administration of anthracyclines by repeated small doses results in lower levels of drug in cardiac tissues compared to the same total dose given as a single bolus (8). Legha et al (5) have recently reported reduced cardiotoxicity in cancer patients who had received DOX as a prolonged continuous infusion. These authors speculated that this lower toxicity was due to a reduction in peak plasma levels of DOX during infusion as opposed to those associated with standard bolus therapy. Similar studies using DNR in patients with leukemia have not been reported.

The in vitro uptake of DNR and other anthracyclines by leukemic cells has been reported by several investigators (9,10). Maximal intracellular DNR concentrations are achieved between 2 and 4 hours after continuous in vitro drug exposure. Similarly, the in vitro uptake patterns of the cells isolated from our patient before each regimen demonstrated maximal DNR uptake after 4 hours of exposure. Based on these in vitro findings, we elected to give a small bolus dose followed by a 4-hour continuous infusion to maximize DNR uptake by the leukemic cells in vivo. Clearly, the cellular uptake pattern during DNR infusion mimics that seen in vitro.

In conclusion, we have demonstrated that a reduction in peak plasma levels of DNR can be achieved without altering intracellular drug concentrations by using a different method of DNR administration.

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Phase II Study of an Aziridinylbenzoquinone (AZQ) in Disseminated Malignant Melanoma[1]

E. T. Creagan,* A. J. Schutt, D. L. Ahmann, and S. J. Green[2]

Thirty-four patients with advanced malignant melanoma received AZQ (an aziridinylbenzoquinone) at a dose of 22.5 or 27.5 mg/m^2/month iv. Objective regressions among 23 previously untreated and 11 previously treated patients were two and none, respectively. Hematologic toxicity was dose-limiting. [Cancer Treat Rep 66:2089–2090, 1982]

Clinically meaningful chemotherapy for patients with advanced malignant melanoma remains elusive. Dacarbazine and the nitrosoureas achieve response rates of only 15%–20% (1,2). Combination regimens have not consistently enhanced either the probability or the duration of objective regressions (1). In this setting, AZQ, an aziridinylbenzoquinone, seemed to be appropriate for clinical trials in advanced melanoma. In animal tumor models, striking activity was noted in an array of solid neoplasms, including Walker carcinosarcoma 256, adenocarcinoma 755, and Sarcoma 180, and in L1210 leukemia (3). In the B16 melanoma murine tumor system, AZQ produced a 140% increase in survival compared with controls (4). Of particular interest are data from primates clearly showing that AZQ has a prolonged concentration in cerebrospinal fluid comparable to plasma levels (5). This is an attractive characteristic of AZQ in light of the alarming frequency of CNS metastases from melanoma.

From a phase I study of our institution, the dose-limiting toxicity was myelosuppression, with clinically acceptable gastrointestinal toxicity. A dose of 27.5 mg/m^2/month produced erratic, occasionally cumulative myelosuppression, especially in patients with prior chemotherapy and/or radiation therapy. For the latter group, a dose of 22.5 mg/m^2/month was deemed appropriate. We report here a phase II study of AZQ in patients with advanced malignant melanoma.

MATERIALS AND METHODS

Thirty-four patients with biopsy-confirmed disseminated malignant melanoma participated in the clinical trial. There were 23 males and 11 females, with a median age of 49 years. Eleven patients had received prior chemotherapy and 23 had had no prior systemic cytotoxic treatment. Eastern Cooperative Oncology Group performance scores (PS) were 0 or 1 (29 patients) or 2 or 3 (five patients). Nineteen patients had visceral-dominant disease, including five with known or suspected liver involvement. Fifteen patients had soft tissue or nodal involvement.

Drug Administration

AZQ was administered iv over 10–15 minutes at a dose of 27.5 mg/m^2 every 4 weeks for the 14 good-risk patients ($<$ 70 years old, PS $\leq$ 2, no prior chemotherapy, and no known or suspected liver metastases). An AZQ dose of 22.5 mg/m^2/month iv was used for poor-risk patients ($\geq$ 70 years old, debilitated, prior chemotherapy, or liver involvement).

Evaluation

Prior to initial treatment and subsequent courses, a thorough general medical assessment, routine tests of hematologic and chemical parameters, chest X-ray, and photographs of appropriate lesions were done for each patient.

An objective response was defined as a decrease $\geq$ 50% in the product of the longest perpendicular diameters of the most clearly measurable indicator lesion. There must have been no new areas of malignant disease or symptomatic deterioration. Progressive disease was defined as a $>$ 25% increase in any indicator lesion or the appearance of new lesions. We analyzed eight patients with evaluable but not clearly measurable dis-

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[1] Received June 1, 1982; revised Aug 16, 1982; accepted Sept 21, 1982.

[2] Division of Medical Oncology (*E. T. Creagan, A. J. Schutt,* and *D. L. Ahmann*), and Cancer Center Statistics (*S. J. Green*), Mayo Clinic, Rochester, MN.

**Reprint requests to:* E. T. Creagan, MD, Division of Medical Oncology, Mayo Clinic, Rochester, MN 55905.

ease using previously reported criteria showing that "assessable but nonmeasurable disease" can provide reliable and consistent response and survival data (6). We calculated the time to disease progression from the first day of treatment to date of progression. Seven patients did not return for evaluation after the first treatment; local physicians confirmed progression of disease in six of these patients, and one patient was assumed to have had progressive disease on the date of scheduled return.

TABLE 1.—Results of treatment by prior chemotherapy exposure and site of metastasis*

Treatment	Visceral-dominant	Nonvisceral
Primary	0/14	2/9
Secondary	0/5	0/6

*Values = No. of objective regressions/No. of evaluable patients.

RESULTS

Therapeutic (table 1).—Among 23 patients with no prior chemotherapy, one experienced a partial regression of nodal and subcutaneous nodules (time to progression, 3.5 months) and one had a complete response of cervical and axillary lymphadenopathy (7 months) (table 1). These two patients were among our 14 "best-risk patients" (no prior chemotherapy, PS of 0 or 1, < 70 years old, and no liver involvement). For all 34 study participants, the median times to progression and survival were 1 and 5 months, respectively (Kaplan-Meier method).

Toxicity.—Toxic effects were primarily hematologic. Among 26 patients with interim blood tests, four (15%) had wbc counts $\leq 1.5 \times 10^3$ cells/mm^3. The median wbc count nadir was 4.0×10^3 cells/mm^3 (range, 1.1–6.5). Six patients (23%) with interim platelet counts had thrombocytopenia ($< 75 \times 10^3$ cells/mm^3). The median platelet count was 150×10^3 cells/mm^3 (range, 33–370). There were no significant episodes of sepsis or hemorrhage. Mild-moderate and transient nausea and vomiting affected 10% of 34 patients; two other patients experienced severe nausea and vomiting. Other toxic effects included alopecia (one patient with mild, one complete), stomatitis (two with mild-moderate), and constipation (five with mild-moderate).

DISCUSSION

In this phase II study of AZQ in advanced malignant melanoma, we achieved a response rate of 9% with acceptable toxicity among 23 patients without prior chemotherapy. A 95% confidence level for the probability of response to AZQ in patients with no prior treatment is 0.01, 0.28. The observed responses were confined to soft tissue or nodal sites, were of marginal benefit, and had no impact on visceral metastases. Therefore, it seems unlikely that AZQ, in the dose and schedule that we used, will meaningfully alter the presently inexorable course of disseminated melanoma. The absence of antitumor activity among 11 previously treated patients is consistent with findings by other investigators who observed only a 2% response rate from secondary chemotherapy in advanced melanoma (1). No responses among 11 patients is inconsistent, at the 0.05 level, with a true response probability of > 25% from AZQ in this patient population.

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Phase II Trial of PALA in Hypernephroma and Urinary Bladder Cancer[1,2]

Ronald B. Natale, Alan Yagoda,* David P. Kelsen, Richard J. Gralla, and Robin C. Watson[3]

PALA, a transition-state analog for aspartate transcarbamylase and a potent inhibitor of de novo pyrimidine biosynthesis, demonstrated antitumor activity against several experimental murine solid tumor models, including the FANFT carcinogen-induced bladder carcinoma (1,2). In the FANFT model, PALA was effective alone, and showed marked synergism when combined with cisplatin (2). In phase I studies, PALA given in a weekly schedule was nonmyelosuppressive, produced manageable gastrointestinal (mucositis/diarrhea) and cutaneous (dermatitis) toxic effects, and induced two minor responses in patients with advanced refractory bladder cancer (3). Therefore, a disease-oriented phase II trial of PALA in patients with objectively measurable metastatic urogenital (bladder or renal) cancers was initiated and forms the basis of this report.

MATERIALS AND METHODS

Thirty-three patients (18 with bladder cancer and 15 with hypernephroma) received PALA. Eligibility criteria included histologically confirmed and bidimensionally measurable metastatic disease, Karnofsky performance status (KPS) $\geq 50\%$, wbc count $> 4000/\text{mm}^3$, platelet count $> 100{,}000/\text{mm}^3$, serum bilirubin < 2.0 mg/100 ml, no chemotherapy in the preceding 3 weeks, and informed consent. A normal electroencephalogram and brain scan by radionuclide or by computerized tomography (CT) were required of the last 14 patients prior to protocol because brain metastases seemed to be a risk factor for PALA-induced CNS toxicity (4). Initially, all patients had a complete history and physical examination, an automated blood cell and platelet count, a 12-channel biochemical screening profile, a serum creatinine (Cr), and a chest roentgenogram. Serum carcinoembryonic antigen (CEA) levels were obtained in 27 patients (17 with bladder cancer and ten with renal cancer), radionuclide liver scans were performed in patients with malignant hepatomegaly and/or abnormal liver function tests, and CT scans were used to evaluate objectively abdominal and pelvic masses. Physical examination and blood cell and platelet counts were performed every week; chest roentgenograms, liver function tests, and serum CEA levels (if elevated) were performed every 3–4 weeks; and liver and abdominal/pelvic CT scans were performed every 4–6 weeks. Patient characteristics are shown in table 1. There were 25 males and eight females, with a median age of 61 years (range, 22–73) and a median KPS of 60% (range, 50%–100%). Except for a patient with bladder cancer, all were quite heavily pretreated, having received a median of three drugs (range, one to five) prior to pro-

TABLE 1.—Patient characteristics

	Bladder cancer	Hypernephroma
Total No. of patients/ No. evaluable	18/17	15/15
KPS		
Median	60%	60%
Range	50%–90%	50%–100%
No. of patients with—		
Complete remission/partial remission	0	0
Minor response	2	0
Prior chemotherapy/hormones	17	7
Prior irradiation	14	3
Prior surgery	10	13
No. of measurable sites		
Lung	9	11
Nodes	7	2
Abdomen/pelvis by CT scan	8	3
Liver	3	2
Skin	1	1
CEA (> 5 mg/100 ml)	9	0

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[1] Received Aug 5, 1982; revised Sept 9, 1982; accepted Sept 28, 1982.

[2] Supported in part by contract N01-CM-97274 (Division of Cancer Treatment) and Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and the Aaron Miller-Solid Tumor Service Fund.

[3] Solid Tumor Service (*R. B. Natale*, *A. Yagoda*, and *D. P. Kelsen*), Developmental Chemotherapy Service, Department of Medicine (*R. J. Gralla*), and Department of Diagnostic Radiology (*R. C. Watson*), Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Alan Yagoda, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

tocol. All patients had bidimensional, objectively measurable disease and 13 (eight with bladder cancer and five with renal cancer) had more than one measurable disease parameter for response evaluation.

PALA was administered iv every week as a 45–60-minute infusion. The initial dose was 4.5 g/m^2 for patients with a KPS $\geq$ 70% and normal renal function and 3.75 g/m^2 for patients with a KPS of 50%–60% or minimal renal dysfunction (Cr, 1.9–3.0 mg/dl or a urinary Cr clearance > 50 ml/minute). The dose was escalated by 20% when toxicity was not encountered after three consecutive weekly doses. When severe toxicity occurred, therapy was discontinued until toxicity resolved, and later resumed at a 20%–50% dose reduction. An adequate trial required three doses of PALA with some evidence of toxicity, or two doses with at least moderate toxicity ($\geq$ 3+), as previously defined (3), and 1-month survival. Partial remission was defined as a > 50% decrease in the summed products of the longest perpendicular diameters of all measurable lesions for > 1 month, minor response was defined as a 25%–50% decrease in measurable lesions, stable disease was defined as a < 25% change for $\geq$ 3 months, and disease progression was defined as a > 25% increase or the appearance of new lesions. KPS was used to evaluate subjective changes.

RESULTS

There were no major objective responses in 32 adequately treated and evaluable patients (17 with bladder cancer and 15 with renal cancer). A minor response, consisting of a 30% decrease in metastatic lung nodules, occurred after three doses in two patients with bladder cancer. However, one of these patients developed PALA-related CNS toxic effects (seizures) which required discontinuation of treatment. He died of progressive disease 2 months later. The other patient died of intracerebral hemorrhaging, probably unrelated to PALA, 5 weeks after starting therapy. One patient with bladder cancer is considered to be unevaluable for response because she received only one dose of 3.75 g/m^2, which resulted in extensive severe cutaneous toxicity leading to sepsis and death 24 days after therapy.

The median number of doses was four (range, one to 16). Thirty-two patients had evidence of cutaneous toxicity, which was moderate or severe (3–4+) in 11. Gastrointestinal toxicity occurred in 17 patients and was generally mild (1–2+). Three patients without preceding abnormal CNS signs or symptoms had the sudden onset of seizures after three, four, and five doses of PALA, respectively; brain metastases were discovered on CT scans in all cases. No CNS toxicity occurred in the final 14 patients entered because of the prescreening procedures previously mentioned. Of note, four patients had moderate to severe cutaneous "flare reactions" in previously irradiated areas, typical of the radiation "recall" phenomena occasionally observed with other chemotherapeutic agents, and two additional patients developed bilateral keratoconjunctivitis and corneal ulcerations.

CONCLUSION

PALA given in a weekly schedule lacks significant antitumor activity in patients with advanced bladder cancer (none of 17 patients; 95% confidence limits, 0%–17%) and renal cancer (none of 15; 95% confidence limits, 0%–19%). Although the extent of prior therapy in our bladder cancer population may have predisposed to drug refractoriness, CNS toxicity associated with a weekly PALA schedule discouraged additional patient accrual. Similar qualifications, however, are not required regarding renal cancer because patients were not heavily pretreated. To date, renal cancer has proven to be unresponsive to PALA when given in a 24–120-hour continuous infusion (three patients), a daily × 5 schedule (three patients), or an every-2-week schedule (one patient) (5–7). Including the results of the present series, there have been no significant responses in hypernephroma in 22 adequately treated cases (none of 22 patients; 95% confidence limits, 0%–13%).

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Combination Chemotherapy in Patients With Advanced Colorectal Carcinoma Previously Treated With 5-FU[1,2]

Harold E. Windschitl,* James E. Krook, Lloyd K. Everson, Stephen A. Cullinan, and Mark Scott[3]

For the North Central Cancer Treatment Group

Semustine, Baker's antifol, and razoxane each have been reported to show activity against colorectal cancer (1–5). For patients failing 5-FU therapy, we elected to study possible combinations of these drugs. We report on a subset of patients entering a clinical trial for advanced colorectal carcinoma conducted by the North Central Cancer Treatment Group.

METHODS

Histologic evidence of neoplastic disease with measurable metastatic lesions, adequate wbc and platelet counts, Eastern Cooperative Oncology Group (ECOG) performance score (PS) < 4, normal nutrition, and previous chemotherapy with 5-FU (on the larger study or not) were required for entry. Prior irradiation or surgery was allowed, but a 4-week interval was required. Patients with elevated bilirubin, cerebrovascular disease, or elevated serum creatinine (limiting conditions) were directly assigned to the semustine plus razoxane program. Patients who had received prior treatment with semustine were assigned to Baker's antifol plus razoxane. Patients having 5-FU, 5-FU plus Baker's antifol, or 5-FU plus razoxane as initial chemotherapy on the larger study, if eligible, were crossed over to razoxane plus Baker's antifol, semustine plus razoxane, or semustine plus Baker's antifol, respectively.

Semustine was given orally at a dose of 150 mg/m^2 on Day 1 regardless of the other drug. Razoxane was given orally at 500 mg/m^2 on Days 2–4 and 29–31 with semustine and at 425 mg/m^2 on Days 1–3 and 29–31 with Baker's antifol. Baker's antifol was given iv over 1 hour at 200 mg/m^2 on Days 1–3 and 29–31 in conjunction with semustine. The dose of Baker's antifol was reduced to 125 mg/m^2 when razoxane was given. Cycles were repeated every 8 weeks. Seventy-seven patients were entered in the study. Two patients were declared ineligible. One patient was not evaluable for response. Table 1 shows patient characteristics. The median age of all patients was 63 years.

RESULTS

Two patients achieved partial response: a crossover patient to Baker's antifol plus razoxane from 5-FU with a PS of 2, as well as a patient with a PS of 0 with limiting conditions who was assigned to semustine plus razoxane. All patients evaluable for response had disease progression and all patients have died. Among randomized patients (as well as a pool of randomized, crossover, and limiting-condition patients) the disease progression times as well as survival times among treatments were not significantly different.

Table 2 shows toxic effects by randomized and nonrandomized patients. Diarrhea was significantly more frequent on one razoxane plus Baker's antifol arm. Both stomatitis and dermatitis were more frequent in the Baker's antifol arms. Thrombocytopenia was more prevalent in the semustine arms.

CONCLUSION

Neither of these two drug combinations offers benefit for the patient with advanced colon or rectal cancer previously treated with 5-FU.

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[1] Received Feb 22, 1982; revised Aug 30, 1982; accepted Sept 13, 1982.

[2] Supported by Public Health Service grant CA-25224 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and American Cancer Society grant ND-104A.

[3] This study was conducted by the North Central Cancer Treatment Group. The following institutions participated in the study: St. Cloud Internists, Ltd, MN (*H. E. Windschitl*); The Duluth Clinic, Ltd, MN (*J. E. Krook*); Fargo Clinic, ND (*L. K. Everson*); Illinois Oncology Research Association, Peoria (*S. A. Cullinan*); Mayo Clinic, Rochester, MN (*M. Scott*); Billings Clinic, MT; Central Plains Clinic, Sioux Falls, SD; Grand Forks Clinic, ND; Iowa Oncology Research Association, Des Moines; and Quain and Ramstad Clinic, Bismarck, ND.

**Reprint requests to:* Harold E. Windschitl, MD, St. Cloud Internists, Ltd, 48 N 29th Ave, St. Cloud, MN 56301.

TABLE 1.—Patient characteristics

Pretreatment characteristics	Semustine + razoxane (% of 27 patients)	Semustine + Baker's antifol (% of 22 patients)	Razoxane + Baker's antifol (% of 26 patients)
Type of trial			
Randomized	67	77	38
Nonrandomized	33	23	62
Sex			
Male	52	27	54
Female	48	73	46
ECOG PS*			
0–1	56	59	58
2–3	44	41	42
Site of primary indicator lesions			
Liver	56	50	50
Lung	36	31	34
Other	8	19	16

*0 = fully active; 4 = 100% bedridden.

TABLE 2.—Toxic effects*

Toxic effects	Semustine + razoxane	Semustine + Baker's antifol	Razoxane + Baker's antifol
Nonhematologic	% of 18 R/ % of 9 NR	% of 17 R/ % of 5 NR	% of 10 R/ % of 16 NR
Nausea	61/67	76/60	70/50
Vomiting	50/67	64/40	50/38
Stomatitis	11/0	35/40	20/13
Diarrhea	17/0	18/0	40/44
Dermatitis	17/0	12/0	20/25
Alopecia	0/0	23/0	10/6
Hematologic	% of 16 R/ % of 7 NR	% of 17 R/ % of 5 NR	% of 9 R/ % of 14 R
Leukopenia (cells/mm^3)			
2000–3999	25/29	47/20	45/50
< 2000	44/29	6/40	11/21
Thrombocytopenia (cells/mm^3)			
50,000–99,999	31/29	29/20	0/21
< 50,000	25/0	12/20	0/7

*R = randomized patient; NR = nonrandomized patient.

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Phase II Study of 5-FU, Doxorubicin, and Mitomycin (FAM) and Chlorozotocin in Advanced Measurable Pancreatic Cancer[1,2]

Frederick P. Smith,* Vinod K. Rustgi, Gerald Schertz, Paul V. Woolley, and Philip S. Schein[3]

The combination of 5-FU, doxorubicin, and mitomycin (FAM) has been demonstrated to be therapeutically active against advanced gastric and pancreatic carcinoma (1,2). For advanced pancreatic cancer, we have previously reported a 37% response rate, with a median response duration of 9 months (2). These results have been corroborated by Bitran et al (3), who obtained responses in five of 14 patients (40%) with pancreatic cancer, with a similar FAM regimen.

Chlorozotocin is a chloroethylnitrosourea with relative bone marrow-sparing properties compared to other compounds in its class (4); this allows it to be incorporated into drug combinations at near full dosage. We designed a phase II trial in which chlorozotocin was added to the FAM regimen to determine whether the combination could be tolerated and whether there were indications of enhanced therapeutic response.

MATERIALS AND METHODS

Between August 1978 and February 1980, 23 patients (13 females and ten males) with advanced pancreatic cancer were entered in the study. A histologically confirmed diagnosis of adenocarcinoma of the pancreas was required, and at the time of initiation of therapy all patients had extensive disease that was not amenable to surgical or radiotherapeutic management. No patient had been previously treated with chemotherapy or radiation therapy. All patients had objective measurable disease by either physical examination and/or special radiologic evaluations. The median age was 60 years (range, 41–76). The performance status (PS) of each patient was assessed at the time of entry in this study, using the Eastern Cooperative Oncology Group system. The median PS was 1, with a range of 0–4.

The FAM-chlorozotocin was administered in 8-week cycles. 5-FU (600 mg/m^2) was administered iv on Days 1, 8, 29, and 36; doxorubicin (30 mg/m^2), on Days 1 and 29; mitomycin (10 mg/m^2), on Day 1; and chlorozotocin (80 mg/m^2), on Day 29. Drug dosage was modified according to the degree of hematologic toxicity, which was measured by wbc and platelet counts as has been previously described for FAM (2).

Durations of response and survival were determined from initiation of FAM-chlorozotocin. A partial response was defined as a decrease of at least 30% in hepatomegaly or a $\geq 50\%$ decrease, lasting at least 4 weeks, in the two largest perpendicular diameters of the most definable lesion, without progression of other disease or clinical deterioration. Stable disease was defined as a $< 50\%$ reduction or a $< 25\%$ increase in tumor size lasting at least 8 weeks.

RESULTS

Three of the 23 patients (13%) achieved a partial response. The median duration of response was 5.5 months (range, 2.5–6.5) from initiation of chemotherapy. Four patients achieved disease stabilization, while 15 had evidence of progressive tumor. The median survival for all patients is 6.4 months (range, 1–15).

FAM-chlorozotocin was well-tolerated. Myelosuppression was the only dose-limiting toxic effect. The median wbc count nadir was $4.0 \times 10^3/\text{mm}^3$ (range, 1.1–7.2), and platelet count nadir was $176 \times 10^3/\text{mm}^3$ (range, 90–500). There were no treatment-related deaths.

DISCUSSION

The FAM-chlorozotocin regimen employed in this phase II study does not have significant activity for

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[1] Received Apr 22, 1982; revised Sept 14, 1982; accepted Sept 22, 1982.

[2] Supported by Public Health Service grant CA-25759 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Medicine, Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Washington, DC.

**Reprint requests to:* Frederick P. Smith, MD, Division of Medical Oncology, Vincent T. Lombardi Cancer Research Center, Georgetown University Hospital, Rm 2100 W, 3800 Reservoir Rd, NW, Washington, DC 20007.

pancreatic cancer. In retrospective comparison to FAM, the addition of chlorozotocin appears to be equally well-tolerated; however, the response rate is lower (37% vs 13%, respectively), and the duration of response is shorter (9 vs 5.5 months, respectively). There were no apparent differences in PS or sites of disease between the groups to explain this discrepancy in responses. The chloroethylnitrosoureas do not have an established role in the management of pancreatic carcinoma. Past trial of carmustine demonstrated no responders among 21 patients, while semustine produced only an 8% response rate (5). Nonetheless, the reason for the discrepancy in responses and, more importantly, in durations of response between FAM and FAM-chlorozotocin is not elucidated by this study. We are not aware that chlorozotocin in other drug combinations has diminished the effectiveness of a standard regimen. However, based on these results we do not recommend further study of this FAM-chlorozotocin regimen for metastatic pancreatic cancer.

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CLINICAL TRIALS SUMMARIES

Phase II Trial of Aziridinylbenzoquinone (AZQ) in Head and Neck Cancer[1,2]

Arlene A. Forastiere,[3,*] Sidney M. Crain,[3] Kent Callahan,[4] David Van Echo,[3] Douglas Mattox,[5] Myo Thant,[6] Daniel D. Von Hoff,[4] and Peter H. Wiernik[3]

1,4-Cyclohexadiene-1,4-dicarbamic acid,2,5-bis(1-aziridinyl)-3,6-dioxodiethyl ester (AZQ) is one of a series of lypophilic aziridinylbenzoquinones that are known to cross-link DNA (1,2). In preclinical testing, AZQ demonstrated a broad spectrum of activity in murine tumor systems (3). In phase I testing, myelosuppression was dose-limiting; gastrointestinal toxicity and transient hepatic dysfunction were infrequent side effects (4). This study evaluated the activity of AZQ in head and neck cancer.

MATERIALS AND METHODS

Twenty-four patients with histologically confirmed epidermoid carcinoma of the head and neck were treated. All patients had measurable disease, a Karnofsky performance score ≥ 50, an expected survival ≥ 8 weeks, a wbc count $\geq 4000/\mu l$, a platelet count $\geq 100{,}000/\mu l$, a creatinine level ≤ 2.0 mg/dl, and a bilirubin level ≤ 1.5 mg/dl. Cbc and platelet counts were repeated weekly, and measurable disease parameters were assessed every 4 weeks.

AZQ was given at a dose of 20 mg/m^2 on Days 1 and 8 every 28 days. On Day 8 the dose was decreased for patients with grades 1, 2, and 3 toxic effects (Cancer and Leukemia Group B criteria) to 15, 10, and 7.5 mg/m^2, respectively. On Day 28 the dose was reduced to 15 mg/m^2 if grade 3 toxicity was reached during the previous cycle. An adequate trial of AZQ consisted of at least two cycles or rapid progression of disease after one cycle at a dose producing a wbc count nadir of $< 3000/\mu l$. Standard response criteria defined in previous trials were used (5).

RESULTS

Eighteen of 24 patients were evaluable for response. There were five early deaths after the first course (three from progressive disease, one from unknown causes, and one from sepsis secondary to drug-induced leukopenia), and one patient was lost to follow-up. Patient characteristics and toxicity to AZQ are detailed in table 1. All patients had had prior radiotherapy (RT), 71% had had surgery in addition to RT, and 66% had received prior chemotherapy. Disease recurred at distant sites in 50% of the patients.

Seventy-two percent of evaluable patients had at least two courses of AZQ. There was one minor response lasting 4 months in a patient with pulmonary metastases; all other patients had disease progression. Nineteen patients were evaluable for toxicity. Dose-limiting toxic effects were hematologic, resulting in dose reduction or treatment delay in six patients. There was one septic death and one life-threatening hemorrhage secondary to drug-induced thrombocytopenia. Hemoglobin dropped ≥ 2.0 g/dl in 12 patients (median, 2.7 g/dl; range, 2.0–6.2) from 1 to 8.5 weeks (median, 4) after the initial dose of AZQ. Progressive decline in hemoglobin was observed with repeated doses. There was no evidence of blood loss or hemolysis, and prior chemotherapy was not associated with increased toxicity.

DISCUSSION

No major responses were observed in this phase II trial of AZQ for advanced epidermoid carcinoma of the head and neck. The median performance status was 70

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[1] Received May 17, 1982; revised Sept 16, 1982; accepted Sept 30, 1982.

[2] Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Medical Oncology Section, University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.

[4] Division of Oncology, Department of Medicine, The University of Texas Health Science Center at San Antonio.

[5] Division of Otorhinolaryngology, Department of Surgery, The University of Texas Health Science Center at San Antonio.

[6] Greater Baltimore Medical Center, MD.

[*] *Reprint requests to:* Dr. Arlene A. Forastiere, VA Medical Center, 2215 Fuller Rd, Ann Arbor, MI 48105.

TABLE 1.—Patient characteristics and drug toxicity

Characteristics	No. of patients
Median age in yrs (range): 55 (22–85)	
Median performance status (range): 70 (50–80)	
Males/females	19/5
Prior treatment	
RT only	1
Surgery + RT	7
RT + chemotherapy	6
Surgery + RT + chemotherapy	10
Primary site	
Oral cavity	3
Oropharynx	10
Larynx	9
Pyriform sinus	2
Sites of measurable disease	
Primary	10
Regional lymph nodes	15
Skin	5
Lung	6
Liver	1
Courses of AZQ	
1	5
2	9
3	3
5	1
Toxicity	
Hematologic	
Wbc count nadir (cells/µl)	
3000–3999	1
2000–2999	4
< 2000	6
Platelet count nadir (cells/µl)	
50,000–100,000	3
< 50,000	3
Hemoglobin decrease (g/dl)	
2–4	9
> 4	3
Nausea	5
Vomiting	1
Stomatitis	1

and all patients except one had been previously treated with two or more treatment modalities. We conclude that in these heavily prior-treated patients, AZQ in this dose and schedule is not a useful agent.

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CLINICAL TRIALS SUMMARIES

Methyl-GAG in Advanced Colon Cancer: A Phase II Trial of the Southwest Oncology Group[1,2]

William A. Knight, III,[3] David M. Loesch,[3] Lawrence P. Leichman,[4] Carol Fabian,[5] and Robert M. O'Bryan[6,*]

Methyl-GAG is a compound with demonstrable activity in acute myelogenous leukemia (1) and lymphoma (2). Prohibitive toxicity with a daily schedule led to the use of an intermittent schedule by the Eastern Cooperative Oncology Group and the Eastern Drug Evaluation Program, resulting in decreased toxic effects (3). In a phase I–II pilot study (4), we utilized a weekly schedule of methyl-GAG at a starting dose of 500 mg/m^2. Acceptable toxic reactions and activity in patients with advanced colon cancer were the basis for this phase II trial.

PATIENTS AND METHODS

Forty patients with pathologically documented colorectal carcinoma and measurable disease were entered in the study. Of these, 36 had received no prior chemotherapy. Other characteristics included a median age of 55 years and a performance status of 1.2 (Southwest Oncology Group criteria).

Methyl-GAG was given as a weekly iv infusion in 100 ml of 5% dextrose in water for $\geq$ 45 minutes. The starting dose of 600 mg/m^2 was increased by 100 mg/m^2/week in the absence of toxic effects. A dose reduction of 100 mg/m^2 was required for grade 2 or 3 toxicity (Southwest Oncology Group criteria), and therapy was interrupted for grade 4 toxicity. Therapy was continued after all toxic effects resolved.

Complete response was defined as a complete regression of all measurable disease; partial response was defined as a $>$ 50% reduction of the product of the two longest perpendicular diameters of the measurable lesions. If hepatomegaly was chosen as a followable parameter, a $>$ 30% decrease in the sum of the measurements below the xiphoid process and both costal margins in the midclavicular line was designated as a partial remission. Patients were considered evaluable for response if they received two courses of methyl-GAG and were followed for at least 4 weeks.

RESULTS

Thirty-two patients were evaluable for response. Seven patients refused further therapy after one course, and one patient died of a myocardial infarction before assessment of measurable disease. Two patients, both with liver metastases, had partial responses of 53 and 29 weeks. Three patients had disease stablization for 43, 12, and 11 weeks.

Toxicity was evaluable for 40 patients and is summarized in table 1. Severe toxic reactions were seen in 14 patients, 13 with severe vomiting and one with hemorrhagic diarrhea. Such toxicity tended to occur after four weekly courses of treatment. Toxicity was reversible when the drug was withheld.

DISCUSSION

Methyl-GAG does not have significant activity among patients with metastatic colon cancer. Prolonged dis-

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[1] Received July 19, 1982; accepted Sept 22, 1982.

[2] Supported in part by Public Health Service grants CA-22433, CA-14028, CA-12644, CA-04915, CA-12014, and CA-32102 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] University of Texas Health Sciences Center at San Antonio.

[4] Wayne State University, Detroit, MI.

[5] University of Kansas Medical Center, Kansas City.

[6] Henry Ford Hospital, Detroit, MI.

*Reprint requests to: Southwest Oncology Group (SWOG-7940), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Toxic effects of methyl-GAG given weekly

Toxic effect	No. of patients
Nausea	26
Vomiting ≤ 6 times/day	7
Vomiting ≥ 7 times/day	6
Hospitalization secondary to vomiting	3
Anorexia	16
Diarrhea	14
3–4 stools/day, no dehydration	6
> 4 stools/day, iv hydration required	3
Bloody diarrhea	1
Mucositis	8
Ulcers, able to eat	3
Ulcers, unable to eat	5
Neuralgia	11
Circumoral paresthesia	13
Balanitis	2
Anemia	15
Decrease in Hgb ≥ 2 g/100 ml	9
Decrease in Hgb ≥ 3 g/100 ml	3
Decrease in Hgb ≥ 4 g/100 ml	3

ease stabilization, seen in a previous study (4), was not observed, and no explanation for this difference is apparent. Most patients in the former study had failed prior chemotherapy, possibly introducing a selection bias between the two studies. Toxicity in this study was more severe than in the previous study. Severe toxicity tended to be cumulative and might be averted by either a rest after four courses or a biweekly schedule. Based on these findings, further trials in colon cancer are not warranted.

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Compilation of 1982 *Clinical Trials Summaries* published in *Cancer Treatment Reports*

Tumor	Drug*	Dosage†	No. of evaluable patients	Response‡	Page
Advanced childhood cancers	Prednimustine	Regimen D: 40 mg/m^2/day for $\geq$ 28 days	99 (all 4 regimens)	3 MR	1583
		Regimen E: 40 mg/m^2/day for 14 days, then 60 mg/m^2/day for $\geq$ 14 days		1 PR (CML), 5 MR	
		Regimen I/200: 200 mg/m^2/day × 5 q 14 days		1 PR (hepatoma), 1 MR	
		Regimen I/300: 300 mg/m^2/day × 5 q 14 days		1 MR	
Breast	AMSA	90 or 120 mg/m^2 by 30-min infusion q 3–4 wks	27	2 MR	403
	AMSA + prednisone + chlorambucil	AMSA: 90 or 120 mg/m^2 iv on Day 1 q 22 days Prednisone: 40 mg/m^2/day orally on Days 1–7 q 22 days Chlorambucil: 0.2 mg/kg orally on Days 1–7 q 22 days	30	NR	1235
	Aziridinylbenzoquinone	30 mg/m^2 by 30-min iv infusion q 3 wks	35	< 7 MR	1875
	Estracyt	560 mg/day orally in 2 divided doses	16	1 PR	581
	Gallium nitrate	700 mg/m^2 in 250 ml of NS over 30 mins following prehydration with 1500 ml of 5% dextrose in $^1/_2$ NS q 2 wks	23	NR	1591
	Spirogermanium	80 mg/m^2 by 1-hr iv infusion on Monday, Wednesday, and Friday for 2 wks, then on Tuesday and Thursday for 2 wks, then once/wk	30	NR	1667
	Tamoxifen	20 mg twice/day (after disease progression during therapy) at 10 mg twice/day	30	NR	1445
	Vinblastine	1.2–1.8 mg/m^2/day by continuous 5-day iv infusion q 3 wks	17	NR	1783
Colorectal	Bisantrene	260 mg/m^2 iv over $\geq$ 60 mins q 3 wks	32	NR	1997
	Cycloleucine	40–50 g/m^2 iv q 3 wks	29	NR	203
	Methyl-GAG	600 mg/m^2/wk by $\geq$ 45-min iv infusion in 100 ml of D5W	32	2 PR	2099
	Mitoxantrone	8–12 mg/m^2 q 3 wks	45	NR	1935
		12 mg/m^2 by 30-min iv infusion in 150 ml of D5W q 3 wks	33	1 PR	1779
	Semustine + razoxane	Semustine: 150 mg/m^2 orally on Day 1 q 8 wks Razoxane: 500 mg/m^2 orally on Days 2–4 and 29–31 q 8 wks	27	1 PR	2093
	Semustine + Baker's antifol	Semustine: 150 mg/m^2 orally on Day 1 q 8 wks Baker's antifol: 200 mg/m^2 by 1-hr iv infusion on Days 1–3 and 29–31 q 8 wks	22	NR	2093
	Baker's antifol + razoxane	Baker's antifol: 125 mg/m^2 by 1-hr iv infusion on Days 1–3 and 29–31 q 8 wks Razoxane: 425 mg/m^2 orally on Days 1–3 and 29–31 q 8 wks	26	1 PR	2093
	Vindesine	2 mg/m^2 iv on Day 1 and 1 mg/m^2 iv on Day 2, q 7 or 10 days	15	NR	583
Endometrial carcinoma	Etoposide	100 mg/m^2 in 150 ml of NS by 45-min iv infusion on Days 1, 3, and 5 q 28 days	29	1 PR	1669
Head and neck					
Squamous cell carcinoma	Bleomycin + hydroxyurea	Bleomycin: 15 units/m^2 iv on Day 1 q 2 wks Hydroxyurea: 1000 mg/m^2/day orally in divided doses on Days 8–15 q 2 wks	19	9 PR	1439
Epidermoid carcinoma	Aziridinylbenzoquinone	20 mg/m^2 on Days 1 and 8 q 28 days	18	1 MR	2097
	Thioproline	40–60 mg/kg/day × 5 q 3 wks	16	1 MR	585

Tumor	Drug*	Dosage†	No. of evaluable patients	Response‡	Page
Leukemia (adult)	AMSA + autologous bone marrow	AMSA: 800 mg/m^2 divided in 4 daily doses Bone marrow: 0.4–1.4 × 10^8 nucleated cells/kg	11	1 CR (AML) 1 PR (ALL) 1 MR (AML)	1991
Lung					
Bronchogenic carcinoma	BCNU + cyclophosphamide + doxorubicin	BCNU: 100 mg/m^2 iv on Day 1 Cyclophosphamide: 400 mg/m^2 iv on Day 1 Doxorubicin: 40 mg/m^2 iv on Day 2	29	1 CR (epidermoid) 1 PR (epidermoid)	199
	Methotrexate	0.6 mg/kg twice/wk	131	5%	197
	Cisplatin + methotrexate + citrovorum factor	Cisplatin: 60 mg/m^2 q 3 wks Methotrexate: 100 mg/m^2 q 3 wks Citrovorum factor: 5 mg/m^2	47	0%	197
	CCNU + cyclophosphamide + methotrexate	CCNU: 50 mg/m^2 orally q 6 wks Cyclophosphamide: 500 mg/m^2 iv q 3 wks Methotrexate: 10 mg/m^2 orally twice/wk	54	15%	197
	Cisplatin + CCNU + cyclophosphamide + methotrexate	Cisplatin: 75 mg/m^2 iv q 6 wks CCNU: 70 mg/m^2 q 6 wks Cyclophosphamide: 700 mg/m^2 q 3 wks Methotrexate: 15 mg/m^2 orally twice/wk	18	11%	197
Small cell	Mitomycin + hexamethylmelamine + vindesine	Mitomycin: 10 mg/m^2 iv on Day 1 q 43 days Hexamethylmelamine: 100 mg/day orally on Days 1–14 and 21–36 q 43 days Vindesine: 3 mg/m^2 iv on Days 1, 8, 21, and 28 q 43 days	13	1 PR	1873
Squamous cell	Vincristine + BCNU + bleomycin	Vincristine: 1.2 mg/m^2 iv at 0 hrs q 2 wks BCNU: 100 mg/m^2 iv at 0 hrs q 2 wks Bleomycin: 5 mg im at 6, 30, 54, 78, and 102 hrs q 6 wks	27	2 MR	1877
Unspecified	DON	160 mg/m^2/day iv × 3 q 3 wks	22	NR	1665
Lymphoma (non-Hodgkin's)	Maytansine	0.5 or 0.75 mg/m^2/wk iv	17	1 PR	1587
Melanoma	AMSA	150 mg/m^2 iv q 21 days	16	NR	1237
	Megestrol acetate	250 mg twice/day orally	20	NR	1239
	Mitolactol	180 mg/m^2/day orally × 10 q 42 days	20	NR	195
	Tamoxifen	10 mg twice/day	23	NR	1771
		10 mg twice/day orally	18	1 PR	1447
		40 mg/day orally in 2 divided doses	21	1 PR	1437
Ovarian	AMSA	90 or 120 mg/m^2 iv over 30 mins q 3 wks	20	NR	1589
	Hexamethylmelamine (or cisplatin) + cyclophosphamide + methotrexate + 5-FU	Hexamethylmelamine: 150 mg/m^2/day orally on Days 1–14 q 4 wks (Cisplatin: 50 mg/m^2 iv on Day 1) Cyclophosphamide: 100 mg/day orally on Days 1–14 q 4 wks Methotrexate: 25 mg/m^2 iv on Days 1 and 8 q 4 wks 5-FU: 300 mg/m^2 on Days 1 and 8	18 (35)	1 PR (5 PR)	1233
	Medroxyprogesterone acetate	500 mg/m^2/day orally in 3 divided doses	27	1 PR	407
		500 mg/day im for 3 wks, then 500 mg once/wk for ⩾ 2 mos	25	1 PR	1441
	Spirogermanium	60 mg/m^2 iv 3 ×/wk during Wks 1 and 2, 60–80 mg/m^2 2 ×/wk during Wks 3 and 4, 60–80 mg/m^2 1 ×/wk at Wk 5 and beyond	13	NR	1675
	Yoshi 864	2.0 mg/kg/day × 5 by slow iv push q 6 wks	33	NR	1775

published in *Cancer Treatment Reports* (continued)

Tumor	Drug*	Dosage†	No. of evaluable patients	Response‡	Page
Pancreatic adenocarcinoma	5-FU + doxorubicin + mitomycin + chlorozotocin	5-FU: 600 mg/m^2 iv on Days 1, 8, 29, and 36 q 8 wks Doxorubicin: 30 mg/m^2 on Days 1 and 29 q 8 wks Mitomycin: 10 mg/m^2 on Day 1 q 8 wks Chlorozotocin: 80 mg/m^2 on Day 29 q 8 wks	23	3 PR	2095
Prostatic carcinoma	*m*-AMSA	100 or 120 mg/m^2 q 3 wks	21	NR	1993
Renal	Dianhydrogalactitol	15, 25, or 30 mg/m^2/day iv × 5 q 4 wks	41	1 PR	1231
	Tamoxifen + medroxyprogesterone acetate	Tamoxifen: 10 mg orally 3 ×/day Medroxyprogesterone acetate: 1.0 g/wk im for ≥ 4 wks	14	NR	1773
	Vinblastine + methyl-GAG	Vinblastine: 5 mg/m^2 by bolus iv infusion on Day 1 q 21 days Methyl-GAG: 500 mg/m^2 by bolus iv infusion on Days 2 and 8 q 21 days	15	NR	1585
	Yoshi 864 + medroxyprogesterone acetate	Yoshi 864: 60 or 80 mg/m^2/day × 5 by iv push q 49 days Medroxyprogesterone acetate: 40 mg/wk im	30	2 PR	1781
Transitional cell carcinoma of urinary tract	VM-26	20 or 30 mg/m^2/day × 5 by 1-hr infusion in 100 ml of NS q 3 wks	40	2 PR	405
Sarcoma	Azapicyl	350 mg/m^2/day orally × 14, 400 mg/m^2/day at Day 14, 450 mg/m^2/day at Day 28	22	NR	399
	Gallium nitrate	700 mg/m^2 by 30-min iv infusion q 2 wks	31	NR	1673
	Prednimustine	25 mg/m^2/day orally	25	1 CR (hemangiopericytoma) 2 PR (fibrohistiocytoma, leiomyosarcoma)	201

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*BCNU = carmustine, CC J = lomustine, VM-26 = teniposide.

†NS = normal saline, D5W = 5% dextrose in water.

‡NR = no response, MR = minor response, PR = partial response, CR = complete response, ALL = acute lymphocytic leukemia, AML = acute myelocytic leukemia, CML = chronic myelogenous leukemia.

LETTERS

Aziridinylbenzoquinone (AZQ) Treatment of Central Nervous System Leukemia

Preclinical and clinical studies have shown that aziridinylbenzoquinone (AZQ) crosses the blood-brain barrier and has antitumor activity against some brain tumors (1-4). Recently we have completed a phase I evaluation of AZQ in pediatric patients with refractory acute leukemia. Doses ranged from 5 to 36 mg/m^2 daily × 5. In this and other studies, AZQ demonstrated antileukemic action (5). Our study included two patients with both bone marrow and CNS leukemia. Both patients showed improvement in the CNS leukemia following iv administration of AZQ.

CASE REPORTS

Patient 1

A $3^1/_2$-year-old white female was diagnosed as having common cell acute lymphocytic leukemia in September 1980. The presenting wbc count was 55,000/mm^3. After achieving a remission with standard chemotherapy, she received intrathecal methotrexate and cranial irradition. Maintenance therapy was 6-mercaptopurine and methotrexate plus 3-day courses of cytarabine and cyclophosphamide every 8 weeks. The patient presented with a CNS relapse approximately 4 months after the cranial irradiation. She had a seizure after the first intrathecal dose of methotrexate and was therefore treated with intrathecal cytarabine and craniospinal irradiation. A bone marrow relapse occurred in October 1981. Treatment with vincristine, prednisone, L-asparaginase, and doxorubicin for 4 weeks failed to produce remission, so the patient was started on teniposide and cytarabine. Two weeks later a spinal tap revealed 1630 cells/mm^3, all blasts. A cbc showed a hemoglobin of 7.8 g/dl; a wbc count of 4200/mm^3, with 3% blasts; and a platelet count of 12,000/mm^3. Bone marrow was M3. The patient was started on AZQ, 24 mg/m^2 daily × 5. One hour after her fifth dose of AZQ, a repeat spinal tap showed only 14 reactive cells/mm^3. The AZQ concentrations in cerebrospinal fluid and in plasma were 120 ng/ml and 200 ng/ml, respectively (courtesy of Dr. John S. Kovach, Mayo Clinic). At that time, hemoglobin was 7.0 g/dl; platelet count, 78,000/mm^3; and wbc count, 900/mm^3 with 1% blasts. Approximately 4 weeks later the patient again developed lethargy and bizarre behavior. A repeat spinal tap revealed 530 lymphoblasts/mm^3. The peripheral blood was still pancytopenic, and a bone marrow sample was hypocellular with residual leukemic cells. A second course of AZQ was given at the same dose. One week after the last dose, the spinal fluid was clear of leukemic cells but was slightly xanthochromic. The patient remained pancytopenic, developed gastrointestinal bleeding and hepatomegaly, and died of gram-negative sepsis 2 weeks later. Post-mortem examination revealed a hypoplastic bone marrow with residual leukemic cells. Focal leukemic infiltrates were seen in the liver and the kidney. Cytocentrifuge preparations of the cerebrospinal fluid contained no lymphoblasts. Brain pathology was not investigated.

Patient 2

A white male, 2 years 10 months old, was diagnosed as having acute lymphocytic leukemia in January 1975. He was started on AZQ in October 1980 after developing a third CNS relapse. Prior pertinent history included testicular disease treated with 2000 rads; bilateral parotid gland infiltrates, also treated with radiation; and five prior bone marrow relapses between March 1978 and October 1980. AZQ (6 mg/m^2 daily × 5) was given during the last week of October 1980. A spinal tap immediately prior to the first dose of AZQ revealed a wbc count of 470/mm^3 with blasts. A repeat spinal tap on the last day of therapy revealed a wbc count of 27/mm^3, with few residual blasts. Neurologic examination was normal. The initial peripheral wbc count was 13,800/mm^3 with 76% blasts. On the last day of therapy the wbc count was 9000/mm^3 with 90% blasts. Two weeks after AZQ the peripheral wbc count was 700/mm^3 with 40% blasts, and a spinal tap showed 240 cells/mm^3. The patient then received intrathecal methotrexate. Because of persistent marrow disease the patient received a second course of AZQ approximately 1 week later. Two weeks after the last dose of AZQ, he died at home of sepsis; he was pancytopenic with residual leukemia. No autopsy was performed.

DISCUSSION

This report demonstrates that systemic AZQ can lower lymphoblast counts in the cerebrospinal fluid. In the first case, response occurred in a patient who had seizures after methotrexate and had recurrences after 5400 rads to the cranium, 1800 rads to the spinal column, and intrathecal cytarabine. The second case also documented a transient response of CNS leukemia in a patient who had had two prior CNS relapses and five bone marrow relapses. In both cases the AZQ produced no signs of CNS toxicity.

Although the incidence of CNS leukemia has been dramatically reduced in children with acute leukemia by the use of CNS prophylaxis, it is still a most difficult problem when it occurs, because the current useful drugs usually fail to eradicate this site of disease and are often associated with severe CNS toxicity. The responses we have seen suggest that AZQ may be a new treatment for CNS leukemia. In these patients, CNS relapse was also associated with bone marrow disease, and therefore, the schedule was designed to treat refractory bone marrow leukemia. A schedule to treat isolated CNS disease has not yet been established. The ability to transiently treat CNS leukemia at even low doses of AZQ warrants more studies and trials of this agent in the treatment of CNS disease.

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Barton A. Kamen
John S. Holcenberg

Midwest Childrens Cancer Center
Milwaukee Childrens Hospital and
Medical College of Wisconsin
1700 W Wisconsin Ave
Milwaukee, WI 53233

Stuart E. Siegel

Childrens Hospital of Los Angeles
Los Angeles, CA 90054

July 6, 1982

Apparent Cyclophosphamide Hypersensitivity: Urticaria Associated With Cyclophosphamide Without Prior Sensitization

Urticaria reaction to cyclophosphamide is so rare that it was not mentioned in recent detailed articles on skin reactions due to chemotherapy (1,2). To date, there are nine reported cases of anaphylactic and urticarial reactions due to iv cyclophosphamide (Cytoxan) (3,4). The triggers of such reactions are not exactly known, and experience with cross-sensitivity to other alkylating agents is not uniform (5,6). With only one exception (7), all previously reported cases either occurred after several weeks or years of treatment with cyclophosphamide or patients had experienced hypersensitivity to other alkylating agents. The present case is unique in that there was no prior exposure to any chemotherapy.

CASE REPORT

A 56-year-old male was diagnosed as having a mucin-secreting adenocarcinoma of the lung in February 1978. He had diffused bony metastasis. Between March 14 and May 8 he received 6000 rads to the lumbar spine to relieve severe pain. He first received chemotherapy on May 30, 1978. The medications consisted of iv cyclophosphamide, doxorubicin, and methotrexate and im prochlorperazine (Compazine). A few minutes later the patient developed a generalized urticaria reaction. He responded promptly to iv diphenhydramine hydrochloride (Benadryl) and methylprednisolone (Solu-Medrol) and was discharged on the same day in good condition. On June 8 and July 3, 1978, the patient received only doxorubicin and methotrexate and, subsequently, doxorubicin, methotrexate, carmustine, and prochlorperazine every 3 weeks for 2 months, without experiencing urticaria reactions.

This is an unusual case in which there was no prior exposure to cyclophosphamide. Urticaria reaction occurred during the first dose of the drug.

It is conjectural whether radiation, which has alkylating properties, could be the sensitizing agent. However, carmustine, which also has alkylating properties, did not produce any hypersensitization effect in our patient or that of Ross and Chabner (5). This experience is also in accord with that of Legha and Hall (6), who found no cross-sensitization between cyclophosphamide, ifosfamide, and mechlorethamine. After a careful study, Lakin and Cahill (8) concluded that cyclophosphamide reaction was mediated by IgE antibody in their patient. No other detailed study has been done to define clearly the mechanism of cyclophosphamide sensitivity. The exact mechanism of cyclophosphamide sensitivity in our patient remains an enigma, since there was no prior exposure to chemotherapy.

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Vincent Anku[1]

Adult Hematology-Oncology
St. John & Westshore Hospital
Campus Medical Arts Bldg #370
29101 Health Campus Dr
Westlake, OH 44145

August 20, 1982

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[1] I am grateful to Dr. John W. Harris for his critique and valuable suggestions.

High-Dose Metoclopramide in Cancer Chemotherapy-Induced Nausea and Vomiting

Nausea and vomiting are among the more frequent and debilitating side effects of cancer chemotherapy. The control of nausea and vomiting induced by highly emetic drugs like cisplatin is known to be problematic (1). Even though conventional doses of metoclopramide have produced controversial effects (1,2), high doses have been found to be well-tolerated and effective (3,4). The preliminary results of a phase II study we have initiated indicate that it is unnecessary to use high doses for every patient and that high-dose metoclopramide can induce more side effects than reported by Gralla et al (3,4), especially in relatively young patients.

There were 33 men and 36 women in the study; all were adults (median age, 55 years). Chemotherapy regimens included cisplatin (75–120 mg/m^2) for 50 patients, dacarbazine (250–400 mg/m^2/day) for 16 patients, and dactinomycin (15 µg/kg/day) for three patients. All these agents can be considered to be highly emetic. Thirty patients had already received one or more courses of chemotherapy. High-dose metoclopramide (HDM) was at first given at a dose of 2 mg/kg in two 1-mg/kg doses. The first dose was an iv infusion over a period of 15 minutes, given 30 minutes before chemotherapy, and the second was given either as a pulse injection 15 minutes after chemotherapy or as a 1- or 4-hour infusion during chemotherapy.

Nineteen patients (27.5%) had no nausea or vomiting, 27 (39.1%) had nausea with perhaps one short episode of vomiting, and ten (14.5%) vomited less than five times over a period of < 3 hours. There was no effect in eight patients (11.6%), and in five patients the effectiveness of HDM was unclear. The antiemetic effect was seen regardless of the chemotherapy used, although it was not as complete when energetic combinations were used (5-FU plus doxorubicin and cisplatin or high-dose cisplatin plus high-dose cyclophosphamide and vindesine). Of the 30 patients who had already received one or more courses of the same chemotherapy, 22 showed a marked antiemetic effect of HDM. Four of 34 patients who received several treatments of chemotherapy with HDM had slight but progressive digestive problems.

Undesirable effects of HDM were seen in 25 patients (36.3%) and consisted of trismus (13 patients); diarrhea (ten); dyskinesia, especially ocular dyskinesia (eight); agitation and anxiety (seven); and drowsiness (three). These effects were considered to be minor in nine patients and troublesome in eight; they required HDM to be stopped in eight of the 25 patients. These effects were always noticed after the first treatment. In four of the eight patients for whom HDM treatment was discontinued, a dose increase to 3–4 mg/kg had been tried. Important side effects of HDM were seen in eight of nine patients younger than age 30.

HDM is a good antiemetic with marked effectiveness for two-thirds of the patients in our series, all of whom received highly emetic chemotherapy. It failed in only 11.6% of the patients. Three points are worth making. First, our results are comparable to those of Gralla et al (3,4), while using one-fifth of the dose of metoclopramide. Second, this decreased dose reduces the administration time of HDM, which makes it compatible with outpatient chemotherapy. Third, even though our doses were much smaller, undesirable effects of HDM were seen in one-third of our patients, which is different from the results of Gralla et al (3,4). Toxic effects were encountered with an unexpected high frequency in young patients, 18–30 years old, who were not considered in the Gralla studies. We therefore feel that further studies are needed, particularly with respect to metoclopramide toxicity, before HDM can be recommended for general use.

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N. B. Bui
G. Marit
B. Hoerni

Fondation Bergonié
F 33076 Bordeaux, France

September 13, 1982

High-Dose Metoclopramide: Control of Dacarbazine-Induced Emesis in a Preliminary Trial[1]

Metoclopramide at high doses given iv has been found to be a safe and effective antiemetic agent (1–4). Double-blind, randomized trials have shown this agent to be superior to placebo (1), prochlorperazine (1), and tetrahydrocannabinol (2) in patients given cisplatin. The following open study was initiated to test the efficacy of metoclopramide in patients receiving dacarbazine, another agent likely to produce emesis.

To date, ten patients receiving dacarbazine at a dose of 1000 mg/m^2 iv over 60–90 minutes have been entered in this study. Patient characteristics included: a median age of 55 years (range, 30–66); a median performance status of 70% (range, 60%–90%); and a diagnosis of malignant melanoma in eight patients, of pancreatic islet cell carcinoma in one, and of soft tissue sarcoma in one. Seven patients had not previously received chemotherapy. The metoclopramide dose of 2.0 mg/kg was given iv over 15 minutes beginning 30 minutes before dacarbazine and was continued every 2 hours for four doses. All patients were observed in the hospital over a 24-hour period and were given no other chemotherapy, with the exception of one patient who also received dactinomycin at a dose of 1.5 mg/m^2 iv.

Nine of the ten patients had major antiemetic responses (0–2 emetic episodes), with four patients having no nausea or vomiting (including the patient receiving dactinomycin with dacarbazine). With this number of patients, the predicted true major antiemetic response rate would be 60%–90% ($P = 0.05$). One patient had a minor antiemetic response (3–5 emetic episodes). The median number of emetic episodes was one (range, zero to five episodes). Only mild side effects occurred, including mild sedation in seven patients and diarrhea (more than three loose bowel movements over 24 hours) in one patient. No extrapyramidal reactions were observed.

The patients entered in this study were all given dacarbazine at a high dose, which generally causes severe emesis (5). With the encouraging results observed in this small number of patients, it appears that larger scale trials with high doses of metoclopramide are indicated in patients receiving dacarbazine.

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Leslie B. Tyson
Rebecca A. Clark
Richard J. Gralla*

Developmental Chemotherapy and Solid Tumor Services
Department of Medicine
Memorial Sloan-Kettering Cancer Center
Cornell University Medical College
1275 York Ave
New York, NY 10021

September 20, 1982

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[1] Supported in part by a grant from the A. H. Robins Co, Public Health Service grant CA-05826, and contract N01-CM-07337 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

*To whom reprint requests should be sent.

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Volume 66
1982

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ANNOUNCEMENTS

Topics in Emergency Medicine

San Francisco, California, January 31–February 4, 1983

This course will be presented by Extended Programs in Medical Education, University of California School of Medicine, San Francisco and will take place at the Sheraton Hotel at Fisherman's Wharf. The program is designed to instruct emergency medical practitioners in depth through didactic presentations, case studies, student/teacher interactions, and demonstrations in the approach to problems in the emergency department. Upon completion of this program, registrants should be more competent in handling injuries of the hand and face, in diagnosing and treating acute cardiac disease, in interpreting radiographs, and in managing problems in other areas including pediatrics, ophthalmology, neurology, obstetrics/gynecology, and endocrinology. Pre and post course assessments will be available, as well as a syllabus with references.

This course meets the criteria for 35 hours of credit in the following: Category 1 of the AMA Physician's Recognition Award, the CMA Certification Program, the American Academy of Family Physicians, and Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, Rm 569-U, Third and Parnassus Ave, San Francisco, CA 94143 (telephone 415-666-4251).

International Lung Cancer Update Conference

New Orleans, Louisiana, March 3–5, 1983

A panel of international lung cancer experts will present the latest information on this worldwide problem, which has now reached epidemic proportions. The meeting is oriented toward an analysis of lung cancer epidemiology and is aimed at gaining insight into prevention and lung cancer risk factors: smoking, industrial exposure, asbestos, nutrition, the interaction of the different factors, etc. This meeting will also include a comprehensive review of the most recent advances in lung cancer biology and the use of this new knowledge to resolve fundamental clinical problems.

This conference has been organized to present the most up-to-date information about these developments to practicing physicians, house staff, nurses, basic scientists, medical and graduate students, and all health professionals involved with cancer patients.

For additional information, contact: Dr. Merle Mizell, Laboratory of Tumor Cell Biology, Tulane University, New Orleans, LA 70118.

Third Annual Current Approaches to Radiation Oncology, Biology, and Physics

San Francisco, California, March 9–11, 1983

Through a syllabus and a series of lectures, this course will include present knowledge and advances in the field of radiation oncology, biology, and physics. The program will be of interest to everyone in the field including students. Presentation of the annual Franz Buschke Prize and the Franze Buschke Lectureship, featuring Dr. Herman D. Suit of Harvard Medical School, will highlight the course.

This program, presented by the Department of Radiation Oncology of the University of California School of Medicine in San Francisco, is sponsored by Extended Programs in Medical Education. The course will meet the criteria for AMA/CMA Category 1 (formal) continuing education credit for physicians. Continuing education credit for nurses and radiation technologists will also be provided.

For further information, contact: Extended Programs in Medical Education, University of California School of Medicine, Rm 569-U, Third and Parnassus, San Francisco, CA 94143. Telephone (415) 666-4251.

Pulmonary Diseases and Critical Care Medicine: A Practical Approach

Park City, Utah, March 12–19, 1983

This program is designed for general internists, family practitioners, general practitioners, pulmonologists, and anesthesiologists. Much of the information covered will also be valuable to physician's assistants, nurses, and respiratory therapists.

The purpose of the program is to provide a practical approach to the evaluation and management of patients with pulmonary diseases, including those who are critically ill.

There will be five sections: environmental and immunologic lung diseases, infectious lung diseases, applied respiratory physiology, diseases of the airways and pulmonary vasculature, and acute respiratory failure. The sections consist of four lectures, followed by a panel discussion and question-and-answer period with all the faculty. The informal atmosphere of the program and the intense participation of the faculty should provide a valuable educational experience for all who attend. A syllabus outlining the lecture topics and containing review articles will be provided at the program.

Program participants will learn to use simple pulmonary function tests in diagnosing and caring for patients, differentiate the various kinds of pulmonary edema and treat them appropriately, apply new antibiotics and bronchodilator drugs, evaluate patients with asbestosis and other pneumoconioses, and manage patients with acute respiratory failure in an intensive care setting.

This program meets the criteria for 9 hours in Category 1 of the Physicians Recognition Award of the AMA and the Certification Program of the CMA, 19 prescribed hours of the American Academy of Family Practice, and 19 hours in Category 1 of the American College of Emergency Physicians.

For further information, contact: Extended Programs in Medical Education, University of California, 1456 Ninth Ave, San Francisco, CA 94122. For registration information, call (415) 666-5808. For program information, call (415) 666-4251.

Third EORTC Breast Cancer Working Conference

Amsterdam, The Netherlands, April 27–29, 1983

This conference will be held at the International Congress Centre in Amsterdam. The intention of the conference is to review a[illegible] discuss topical subjects and matters of controversy in the treatment and research into the biology of breast cancer with the hope of defining future lines for investigations.

Review lectures, workshops, and poster/slide sessions will be held on the following topics: biology of the breast; receptors; epidemiology, methodology, and statistics; diagnosis and screening; psychosocial aspects; adjuvant therapy; markers and prognostic factors; benign vs. malignant proliferative lesions; breast-conserving therapy; and treatment of advanced disease.

The conference language will be English; presentation of papers and discussions in this language is obligatory.

Participants will receive a free copy of the abstracts at the meeting. The review lectures and reports of the workshops will be published in an issue of the European Journal of Cancer.

Participants are encouraged to register before March 1, 1983.

For further information, contact: Dr. J. A. Van Dongen, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Telephone 31–20–5129111.

Predictive Drug Testing on Human Tumor Cells

Zurich, Switzerland, July 20–22, 1983

An international conference on predictive drug testing on human tumor cells will be held at the University Hospital of Zurich. The conference will deal with newly developed techniques for individualizing treatment of cancer patients. The program will include lectures by invited speakers, competitive papers, and a poster session. The deadline for receipt of abstracts is April 15th, 1983.

For further information, contact: V. Hofmann, MD, Division of Oncology, University Hospital, 8091 Zurich, Switzerland.

International Congress on the Basic and Clinical Aspects of the Endocrinology of the Breast

Torino, Italy, September 1984

The University of Torino, Italy, and the New York Academy of Sciences are sponsoring an international Congress to be held in Torino in September 1984. The aim of the meeting is to review the knowledge of the endocrine control of the breast, with emphasis on hormone interactions, breast biochemistry and physiology, benign breast disease, and cancer risk.

For further information, contact: Alberto Angeli or Luigi Dogliotti, Patologia Speciale Medica D, Università degli Studi di Torino, Via Genova 3, 10126 Torino, Italy.

☆U.S. GOVERNMENT PRINTING OFFICE: 1982 361-160/6 1-3

a publication
of the
national cancer institute

NIH Publication No. 83-22